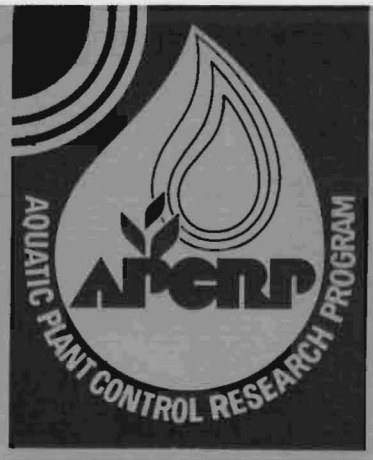

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ENVIRONMENTAL FATE, EFFECTS, AND HEALTH HAZARDS OF FENAC

By Harish C. Sikka, Edward J. Pack,
Henry T. Appleton, Robert Hsu, David Cunningham

Syracuse Research Corporation
Merrill Lane
Syracuse, N. Y. 13210

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Various environmental factors (e.g. hydrolysis, photodegradation, microbial degradation, sorption to sediment, and bioaccumulation by aquatic organisms) were investigated that may influence the fate of fenac in the aquatic environment as well as its effect on certain aquatic organisms. To assess the potential hazard of fenac to human health, the metabolic fate of the herbicide in

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rats and its ability to induce mutations also were examined.

In these studies it was found that fenac was not adsorbed in significant amounts by aquatic sediments nor did it undergo hydrolysis in an aqueous solution. The herbicide was not readily degraded in an aqueous solution by light at 300 nm, but did undergo photodegradation in the presence of certain photosensitizers. The microorganisms in lake water, sediment, or activated sludge were not able to degrade the herbicide to a significant extent.

Fenac did not bioaccumulate in fish (bluegill sunfish and catfish) or Daphnia to a significant extent and was not metabolized by the fish.

Fenac was readily adsorbed by rats following oral and intraperitoneal administration, metabolized to several products and the chemical and its metabolites were eliminated from the animal via both the urine and feces. The major route of elimination of the fenac and its metabolites was via the urine. Although fenac and its metabolites were distributed to a variety of tissues, the major sites of distribution were the lung, liver, and kidney.

Fenac was not active in inducing mutations in Salmonella typhimurium (Ames test).

PREFACE

The study presented herein was sponsored by the Aquatic Plant Control Research Program (APCRP) of the Civil Works Directorate of the Office, Chief of Engineers (OCE). Funds for the study were provided by the Directorate of Civil Works, OCE, Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed by the U.S. Army Engineer Waterways Experiment Station (WES), CE, Vicksburg, Miss.

During the 3-year study, several people had significant roles in the conduct of this research and preparation of the report: Dr. Harish Sikka, Project Director; Dr. Henry Appleton, toxicity and bioaccumulation of fenac in aquatic organisms; Dr. Robert Hsu, pharmokinetics and metabolism of fenac in rats; Mr. Edward Pack, hydrolysis, photodegradation, sediment sorption, and biodegradation of fenac; and Mr. David Cunningham, biodegradation of fenac in activated sludge, effect of fenac on microbial activities, and mutagenicity assays.

The study was conducted under the general supervision of Dr. John Harrison, Chief, Environmental Laboratory (EL), WES; Dr. R.L. Eley, former Chief, Ecosystem Research and Simulation Division (ERSD), EL; Mr. Donald L. Robey, Chief, ERSD; Dr. R.M. Engler, Chief, Ecological Effects and Regulatory Criteria Group (EERCG), ERSD; and under the direct supervision of Dr. Howard Westerdahl, EERCG. Mr. J.L. Decell is Manager of the APCRP at WES.

Commanders and Directors of WES during the conduct of this study and preparation of the report were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F.R. Brown.

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PART I: INTRODUCTION

BACKGROUND

1. The herbicide fenac, 2,3-6-trichlorophenylacetic acid, has been found to be effective against submerged aquatic weeds including Hydrilla (5,6,7,8). The introduction of fenac into the aquatic environment for controlling aquatic plants is of environmental concern because of its potential toxicity to aquatic fauna and flora and its possible adverse effects on man through contamination of his drinking-water supplies. Also, the herbicide and its degradation products may accumulate in fish and could pose a health hazard if fish from contaminated waters were to be used as human food. Furthermore, fenac may be converted to other products as a result of biological or nonbiological transformation in the aquatic environment. Since the transformation products may be more toxic and/or more persistent than the parent chemical, they may present additional pollution problems. Therefore, in order to fully evaluate the hazards associated with the use of fenac in the aquatic environment, it becomes important to study the environmental fate of the herbicide since its persistence, disappearance, or partial transformation will determine the degree of its hazardousness. Several physical, chemical, and biological factors determine the fate of a chemical in the aquatic environment. These include photodegradation, chemical hydrolysis, adsorption to sediment, microbial degradation, and bioaccumulation by aquatic organisms. Currently, very little is known about the effects of these factors on the persistence of fenac in the aquatic environment. This study was undertaken to assess the role of some of the processes which may determine the environmental behavior of fenac.

2. In order to assess the toxic potential of fenac to mammals, it is essential to have a knowledge of its behavior in the body (e.g. absorption, excretion, concentration in specific tissues, and metabolism). Since virtually nothing is known about the metabolic fate of fenac in mammals, the authors investigated the disposition and metabolism of the herbicide in rats. The authors also tested the herbicide for its ability to induce mutations in Salmonella typhimurium (Ames test) to assess its potential hazard to human health.

OBJECTIVES

3. The overall objective of this investigation was to obtain information needed for registration of fenac for aquatic plant control.

4. A number of studies were undertaken in an attempt to obtain information on the environmental behavior of fenac and its potential hazard to human health. The studies were designed (a) to determine the sorption of fenac by aquatic sediments; (b) to study the hydrolysis of fenac in aqueous solution; (c) to examine the photodegradation of fenac in aqueous solution; (d) to study the biodegradation of fenac in lake water; (e) to assess the effect and biodegradation of fenac in activated sludge; (f) to investigate the effect of fenac on the activity of aquatic microorganisms in aquatic sediment; (g) to assess the effect of fenac on fish and Daphnia; (h) to determine the uptake and metabolism of fenac by fish and Daphnia; (i) to determine the absorption, excretion, and tissue distribution of fenac in rats; (j) to study the in vivo and in vitro metabolism of fenac in rats; and (k) to assess the mutagenic activity of fenac in Salmonella typhimurium (Ames test).

PART II: ENVIRONMENTAL FATE AND EFFECTS OF FENAC

MATERIALS AND METHODS

Chemicals

5. Uniformly ring-labelled ^{14}C-fenac (2,3,6-trichlorophenyl acetic acid with specific activities of 3.8 and 29 mCi/mMole) was purchased from California Bionuclear Corporation, Sun Valley, California. The radiochemical purity of this chemical was >97 percent, determined by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

6. Technical grade fenac (70.9 percent 2,3,6-trichlorophenyl acetic acid) was provided by Amchem Products. This chemical is a mixture of trichlorophenyl acetic acid isomers in which the 2,3,6-trichloro isomer constituted 71 percent. It was purified twice by recrystallization from hot benzene. The melting point of the purified fenac was found to be 154-157°C. The recrystallized material contained about 91 pecent of the 2,3,6-trichloroisomer as shown by gas-liquid chromatographic analysis of the fenac. The recrystallized fenac exhibited one major peak (2,3,6-trichlorophenyl acetic acid) along with two minor ones which corresponded with the other trichloroisomers, the identity of which cannot be established on the basis of mass spectral analysis.

Analytical Methods

7. The analytical methods used to determine fenac and its degradation products included gas-liquid chromatography (GLC), high-pressure liquid chromatography (HPLC), and radioassays.

Gas-liquid chromatography

8. The water samples were acidified to approximately pH 2 and extracted twice with diethyl ether. The combined ether extract was concentrated to about 0.1 ml and the free fenac acid was converted to its methyl ester using diazomethane (19,20). The methyl ester was analyzed using a Hewlett

Packard Model 5840 GLC equipped with an electron-capture detector and a column containing 3 percent OV-I on 100/120 mesh Supelcoport. The operating GLC conditions were as follows; 50 m/min of N_2 carrier gas; injector, column, and detector temperatures of 200°, 165°, and 325°, respectively.

High-pressure liquid chromatography

9. Analysis was performed using a Waters Associates HPLC (Model 6000A) equipped with a Schoeffel SF770 UV detector and a μ-Bondapak C_{18} reversed phase column. Prior to HPLC analysis, the water samples were mixed with acetonitrile (1:1) and filtered through a milipore 0.5-μ teflon membrane filter. The column was eluted with acetonitrile:4 percent acetic acid (60:40). Fenac was detected by its absorbance at 225 nm. HPLC proved to be the preferred method of analysis of fenac because it permitted direct determination of the herbicide in an aqueous solution, thus eliminating the need for extraction from water and subsequent derivitization prior to GLC analysis.

Radioassays

10. All radioassays were performed using a Packard Model 3255 Tri-Carb scintillation counter with automatic external standardization. The ^{14}C samples were combusted in a Packard Tri-Carb Sample Oxidizer, Model 306. ^{14}C-fenac and metabolites eluting from the HPLC column were detected and quantified by a radioactive flow detector (Radiometric Instrument and Chemical Company). Thin-layer chromatograms were scanned for radioactivity using a Nuclear Chicago Actigraph.

Laboratory Tests

Octanol-water partition coefficient

11. Octanol-water partition coefficient of fenac was determined according to Leo et al. (6) with slight modifications. The ^{14}C-fenac, dissolved in methanol, was added to stainless steel centrifuge tubes, the

methanol was evaporated, and known volume of water-saturated octanol was added to each tube. After fenac was dissolved, a known volume of octanol-saturated water was added, and the tubes were sealed and shaken until equilibration was achieved (first determination) as measured radiometrically after centrifugation for 30 minutes at 10,000 rpm (25°C). At this point, additional octanol-saturated water was added to disrupt equilibration and the procedure repeated (second determination). The partition coefficient was calculated as the ratio of the concentration of fenac in octanol to that in water.

Photodegradation of fenac

12. The photodegradation of fenac in an aqueous solution was examined following irradiation with simulated sunlight. A solution of fenac in distilled water was irradiated with a 450-watt Hanovia high-pressure mercury vapor lamp in a photochemical reactor manufactured by the Ace Glass Company. The reaction system consisted of a jacketed borosilicate glass vessel equipped with a side arm for withdrawing samples. The double-walled, water-cooled quartz well, housing the light source, was fitted into the vessel and immersed in the solution to be irradiated. The lamp was fitted with a Pyrex 7740 that excluded light of wavelength less than 280 nm. Aliquots of the photolyzed solution were withdrawn at appropriate intervals and analyzed for fenac. To characterize the products resulting from the photodegradation of fenac, the photolyzed solution was acidified to approximately pH 2 and extracted twice with diethyl ether. The combined ether extract was evaporated to about 0.1 ml and then methylated using diazomethane (19,20). The methylated extract was analyzed by combined GLC-mass spectrometer (Finnigan 3300 GC/MS/DS) at Cornell University's mass spectral facility, Ithaca, New York.

13. Quantum yield of fenac was measured using a Rayonett Model RMR-400 mini-photochemical reactor.

Hydrolysis of fenac

14. Separate 250-ml samples of distilled water were buffered to pH 5.0 (0.01 M acetate), pH 7.0 (0.01 M phosphate), and pH 9.0 (0.01 M borate) and were autoclaved in Erlenmeyer flasks. Fenac was then added to the flask at a concentration of 2 ppm. The flasks were incubated in the dark at 10° and 22°C in an environmentally controlled chamber and maintained under sterile conditions. Aliquots of solution were withdrawn periodically and analyzed for fenac using the HPLC procedure described earlier.

Adsorption of fenac by sediment

15. These studies were done using four types of sediments (organic muck, reduced clay, oxidized clay, and sandy sediment) provided by the U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. The sediments were passed through a screen to remove pebbles, leaves, and other detritus. To determine adsorption, 0.5 g sediment (dry-weight equivalent) and 50 ml of a 2 ppm solution of ^{14}C-fenac were added to an Erlenmeyer flask. The pH of the sediment suspension was 6.5. Each flask was shaken at ambient temperature (22°C) in the dark. Following the equilibration period (determined by measuring the disappearance of ^{14}C from the solution at various intervals over 24 hours), aliquots of the suspension were centrifuged at 12,000 x g and the clear supernatant was counted for ^{14}C. The amount of ^{14}C-fenac disappearing from the solution was assumed to be sorbed by the sediment. This amount was corrected for any loss of the ^{14}C-chemical due to sorption on the glassware. The degree of sorption was expressed as distribution coefficient (Kd), the ratio of the amount of fenac adsorbed to the amount in equilibrium solution.

16. The effect of fenac concentration on the degree of sorption was measured for the organic muck. The sediment was suspended in solution containing varying concentrations of fenac and sorption was determined as described previously.

Biodegradation of fenac in lake water and sediment

17. Four 19-ℓ glass aquaria containing 6 ℓ of the lake water and a 5-cm layer of sediment were placed in a constant temperature (18° ± 1°C) chamber. The same four types of sediments employed in the adsorption experiments were used in these studies. After a 7-day equilibrium period, a stock solution of fenac was added to the aquaria so that the final concentration of fenac in the water was 2 ppm. The fenac solution was added to the water dropwise over a period of two hours to minimize disturbance of the sediment layer. The water was then gently stirred with a glass rod to ensure an even distribution of the herbicide throughout the water. Duplicate samples of water were taken at appropriate intervals and analyzed for fenac by GLC as described earlier. Distilled water was added to the aquaria weekly to maintain the level of water in the aquaria; fresh lake water was added every four weeks to ensure an active microbial population.

Effect of fenac on aquatic microorganisms

18. The sediment for these studies was collected from Oneida Lake, sieved through a 2-mm mesh sieve, thoroughly mixed, and stored at 4°C in glass jars. The effects of fenac on microbial metabolism included tests for oxygen consumption; carbon dioxide evolution; protein, cellulose, and starch degradation; and nitrification.

19. Oxygen consumption. The rate of oxygen consumption was measured with a Warburg respirometer by conventional techniques (18). Each Warburg flask contained 4 g sediment (2 g dry weight) to which technical grade

fenac was added from an aqueous stock solution to give final concentrations in the water of 2 and 10 ppm of the herbicide. Each treatment included two replications. The flasks were incubated with shaking on the respirometer at 20°C for 8-hour periods during each day when measurements were made. When oxygen consumption was not being measured, flasks were removed from manometers, covered with parafilm to prevent evaporative losses, and incubated at 20°C with shaking.

20. Carbon dioxide (CO_2) evolution. The effect of fenac on CO_2 evolution from unamended sediment was measured in biometer flasks (1). Each flask contained 20 g sediment (10 g dry weight) to which technical grade fenac was added from an aqueous stock solution to give final concentrations of 2.0 or 10 ppm in water. The total water content in each flask was brought to 20 ml with distilled water. Each treatment included two replications. The flasks were incubated on a rotary shaker at 20°C $\pm$ 2° and aerated daily. Carbon dioxide evolution was measured by periodically replacing the 0.1 N potassium hydroxide (KOH) trapping solution and titrating it with 0.05 N hydrochloric acid (HCl).

21. Protein degradation. Sediment was incubated with casein and the rates of CO_2 evolution and nitrate production were observed in biometer flasks (1). Each flask contained 20 g sediment (10 g dry weight) to which technical grade fenac was added from an aqueous stock solution to give final fenac concentrations of 2 or 10 ppm in the water. The total water content in each flask was brought to 20 ml with distilled water. A 20-ml aliquot of casein (Fisher Scientific Co., vitamin free) was evenly dispersed in the sediment of each flask. Each treatment included two replications. The flasks were incubated on a rotary shaker at 20° $\pm$ 2°C and aerated daily.

The CO_2 trapping solution (10 ml, 0.1 N KOH) was periodically removed from the side arm of the flask, replaced, and titrated with 0.05 N HCl to determine the quantity of CO_2 evolved. Nitrate content was measured with an Orion nitrate-specific electrode at time zero and after two weeks incubation.

22. Cellulose degradation. The rate of cellulose degradation in the lake sediment was measured by assaying the $^{14}CO_2$ evolved from ^{14}C-labelled cellulose in biometer flasks (1). Each flask contained 20 g sediment (10 g dry weight) to which technical grade fenac was added from a concentrated aqueous stock solution to give final fenac concentrations of 2 or 10 ppm in the water. The total water in each flask was brought to 20 ml with distilled water. Uniformly labelled ^{14}C-cellulose (20 mg 5 μ Ci) was evenly dispersed in the sediment of each flask. Cellulose was prepared by mixing ^{14}C-labelled cellulose (New England Nuclear Corp.) with unlabelled cellulose (Sigma Chemical Co.). Each treatment included two replications. The flasks were incubated on a rotary shaker at 20° ± 2°C and aerated daily. The side arm of each flask contained 10 ml of 0.1 N KOH to trap CO_2. The solution in the side arm was replaced periodically and assayed for $^{14}CO_2$ by liquid scintillation counting. The radioactivity collected in the KOH trap was verified as $^{14}CO_2$ after acidification with HCl.

23. Starch degradation. Starch degradation in lake sediment was measured by assaying the $^{14}CO_2$ evolved from ^{14}C-labelled starch. The experiments were set up in biometer flasks as in the cellulose degradation study. Each flask contained 20 g sediment (10 g dry weight) to which technical grade fenac was added from an aqueous stock solution to give final fenac concentrations in the water of 2, and 10 ppm. Uniformly labelled ^{14}C-starch (20 mg, 5 μ Ci) in aqueous suspension was mixed into

the sediment in each flask and the total water content was brought to 20 ml with distilled water. The starch suspension was prepared by boiling a mixture of ^{14}C-labelled starch (New England Nuclear Corp.) and nonlabelled potato starch (Sigma Chemical Co.). The evolved $^{14}CO_2$ was trapped in 0.1 N KOH and assayed for radioactivity as described below.

24. Nitrification. The effect of fenac on nitrification in the lake sediment was studied by amending sediment samples with ammonium sulfate ($(NH_4)_2SO_4$) and measuring the amount of nitrate produced using a nitrate-specific electrode. An aqueous solution of $(NH_4)_2SO_4$ containing 2 mg of ammonium nitrogen was added to 20 g of sediment (10 g dry weight) in a 250-ml Erlenmeyer flask. Technical grade fenac was added from an aqueous stock solution to give final fenac concentrations in the water of 2 or 10 ppm. The total water content of each flask was brought to 20 ml with distilled water. The flasks were stoppered with foam plugs and incubated on a rotary shaker in the dark at 20° ± 2°C. The flasks were removed and assayed at various times for nitrate using an Orion nitrate-specific electrode. Each treatment was run in duplicate.

Fate and effect of fenac in activated sludge

25. To examine the fate and effect of fenac in activated sludge, a sludge unit was set up using the Soap and Detergent Association's 24-hour semicontinuous procedure (SCAS) (17) recommended by the Environmental Protection Agency (EPA) guidelines. The unit consists of a cylindrical glass chamber of 1500-ml working volume with provision for aeration, sampling, and draining. The activation sludge culture was obtained from a local municipal sewage treatment plant and acclimated to synthetic sewage (NH_4Cl, 3.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.25 g; KCl, 0.25 g; $FeSO_4.7H_2O$, 0.002 g; yeast extract, 0.3 g; water, 1 ℓ). Prior to conducting the biodegradation studies, the sludge unit was operated for four weeks in order to stabilize the microbial population.

26. The procedure for examining the fate of fenac in the sludge involved adding incremental doses of ^{14}C-fenac to the unit daily. CO_2-free air was bubbled through the mixture daily to provide constant aeration and mixing. The effluent gas was passed through two flasks containing 0.01 N KOH to trap any evolved CO_2. The 1500 ml of sewage and sludge was aerated for 23 hours. The sludge solids were allowed to settle for 20 min and then 1000 ml of the supernatant was removed. The sludge remaining in the test unit was then readjusted to the original volume of 1500 ml by adding fresh synthetic sewage containing ^{14}C-fenac (25 μ Ci/mM) and the cycle was repeated. Fenac was added daily to the sludge in incremental doses according to the following schedule: 0.1, 0.2, 0.4, 0.8, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, and 40 ppm. The CO_2-trapping solution was removed and replaced daily.

27. The effluent (representing treated water from a sewage treatment plant) and settled sludge were assayed for total radioactivity. The amount of ^{14}C in the CO_2-trapping solution was measured by liquid scintillation counting. The total amount of CO_2 evolved was determined by precipitation with barium chloride ($BaCl_2$) followed by titration of the remaining KOH against a standard acid.

28. Aliquots of the effluent and settled sludge were acidified to ∿ pH 2 and extracted with diethyl ether. The ether extract was chromatographed on thin-layer silica-gel plates using a two-solvent system: (a) water-saturated benzene:acetic acid, 4:1, and (b) ethanol:water:aq. ammonia, 16:3:1. The plates were then scanned for radioactivity on a Nuclear Chicago Actigraph. The extracts were also analyzed by high-pressure liquid chromatography as described earlier.

Toxicity of fenac to fish

29. Fathead minnows. Fathead minnows (Pimephales promelas) were obtained from a local baitfish hatchery and acclimated to laboratory conditions. Fenac exposure solutions were prepared by adding a stock solution of purified fenac in methanol to 4-ℓ glass jars. The methanol was evaporated, 2 ℓ of dilution water (dechlorinated and aged municipal tap water) was added to the jars, and the water was agitated until solution was achieved. Five fathead minnows (1.9-3.8 cm length) were added to each jar and aeration was initiated at an approximate rate of 100 bubbles per minute through glass capillary pipets. The treatments included 40, 30, 20, 10, and 5 ppm fenac solutions and controls (only methanol added, no fenac). The treatments were performed in duplicate with five fish per replicate. The pH of the initial solutions ranged from 7.6-7.9 and dissolved oxygen content varied from 7.68-8.3 ppm. The temperature ranged from 22°-23.5°C during the study. The fish were observed frequently during the first 24 hours for symptoms of toxicity, and daily for three additional days. Dead fish were immediately removed. Dissolved oxygen content of the treatments was routinely measured.

30. Rainbow trout. Rainbow trout (Salmo gairdneri), of Plymouth Rock strain (ca. 2 in. (5 cm)) were obtained from Naylor Hatchery, Cazenovia, New York. The trout were acclimated to laboratory conditions for one month at 12°C. To test for acute toxicity of fenac to trout, the fish were placed in solutions of technical grade fenac (ca. 70 percent, 2,3,6-trichloro isomer) prepared in dechlorinated and aged municipal tap water at a loading ratio of 0.7 g fish/ℓ. The temperature was maintained at 12° $\pm$ 0.5°C and solutions were mildly aerated to maintain a dissolved oxygen content of 9.5-10.1 ppm through the course of the experiment. The

initial pH was 7.6. The trout were observed daily for signs of distress or intoxication and were not fed during the 96-hour period.

Toxicity of fenac to Daphnia

31. A synthetic medium was used for mass rearing of Daphnia magna and for conducting bioassays. Each liter of the medium contained KCl, 50 mg; $MgSO_4.7H_2O$, 40 mg; $CaCl_2$, 55.5 mg; K_2HPO_4, 6 mg; $NaNO_3$, 50 mg; $NaSiO_3 9H_2O$, 20 mg; $FeCl_3$, 0.5 mg. The pH of the medium was adjusted to 7.0. Control mortality in this medium consistently averaged less than 5 percent over a 96-hour period without feeding, or over one month with feeding. Purified fenac (2,3,6-trichloro isomer content of 91 percent) was utilized in these studies. Daphnia magna were obtained from a biological supply house and mass reared in the laboratory on a diet of baker's yeast and algae. First instar Daphnia, less than 24 hours old, were obtained from at least two individually reared adults, placed in a single container, and selected at random for testing. All tests were performed at 20° $\pm$ 1°C, with a daily light/dark cycle of 14 hr/10 hr.

32. Acute toxicity. First instar Daphnia (24 hours old or less) were placed in toxicant solution at a loading ratio of one daphnid per 10 ml solution, and observed for symptoms of intoxication and mortality at 6, 24, 48, 72, and 96 hours. Because of the low mortalities observed through 48 hours, acute toxicity was reported for only the 96-hour period. The animals were held without food during the determinations. The mortality of daphnids in controls without fenac at 96 hours ranged from 1.5 to 3.5 percent. The surviving animals were counted at 96 hours. Animals were judged to be alive if they would swim in response to light or physical agitation. The nonswimming animals were assumed to be dead. However, the data are reported as EC_{50} (median effective concentration) due to the difficulty of actually judging death in Daphnia. The percent mortality of

each treatment was calculated by the equation:

$$\text{corrected \% mortality} = \frac{\text{\% mortality treatment} - \text{\% mortality control}}{100 - \text{\% mortality control}}$$

33. Daphnia reproductive effects. One first instar Daphnia was placed in 100 ml of the artifical medium containing fenac. Ten replicates were included in each treatment level. The fenac levels tested included 20 ppm, 5 ppm, 0.5 ppm, and a control without fenac. The Daphnia were fed on alternate days with a mixture of baker's yeast and algae (3).

34. The offspring produced by the control and fenac-treated Daphnia were analyzed in several ways to determine fenac effects on daphnid reproduction. These include: (a) number of offspring per brood, (b) total number of broods in a given time, and (c) total number of offspring produced in a given time or in the lifetime of the parent animal.

Bioaccumulation of fenac by bluegill sunfish

35. Bluegill sunfish (Lepomis macrochirus Raf.) were obtained from the National Fish Hatchery, Orangeburg, S.C., and acclimated to laboratory conditions prior to fenac exposure.

36. Static exposure. The initial uptake study was for five days with seven whole bluegills analyzed daily for fenac. The fish were introduced into water containing 2 ppm ^{14}C-fenac at a specific activity of 0.37 m Ci/mM (3360 disintegrations per minute (dpm)/μg). Each liter of water in the aquarium contained two fish (average loading: 1.5 g fish/liter of water) and the water was continuously bubbled with air during the experiment. After five days, three fish were removed from the test water, rinsed well, and separated into edible flesh (including attached skin and bones) and head plus viscera portions; each portion was homogenized separately with methanol. The homogenates were shaken for one hour, centrifuged, and the extract was decanted. Next, the residue was

extracted with methanol. The two extracts were combined and the amount of radioactivity in the pooled extracts was measured by liquid scintillation counting. The amount of ^{14}C in the tissue residue was determined by solubilizing it in NCS® tissue solubilizer (Amersham Searle Corp.) for 48 hours at 50°C (16). Glacial acetic acid (0.02 ml/ml of solubilizer) was added to the solubilized tissue and the solution was counted for radioactivity in scintillation fluid containing Triton X-100. The radioactivity in the methanol extract and in the tissue was combined to calculate the total ^{14}C level in a tissue.

37. Flow-through exposure. Dilution water was delivered with a peristaltic pump to a mixing chamber into which a syringe pump delivered a concentrated methanol stock solution of ^{14}C-fenac (specific activity 50 dpm/µg). The diluted exposure solution, averaging 1.36 ppm fenac during the study, was conveyed by gravity flow to the exposure aquarium fitted with a stand-pipe drain; each was filled with a 15-ℓ volume, which was replaced five times daily. After a stable concentration of ^{14}C-fenac was attained in the aquarium, 50 bluegills (ca. 1 in. (25.4 mm) length) were introduced into the exposure tank. Duplicate 1-ml aliquots of water were taken daily from the exposure tank and counted for radioactivity. Periodically, the fish were sampled, sacrificed, and analyzed for total ^{14}C as described above. When the ^{14}C-residue in the fish had reached equilibrium, the remaining fish were transferred to clean flowing water for the depuration phase. The fish were sampled at various intervals after start of depuration and analyzed for total ^{14}C content.

Bioaccumulation of fenac by catfish

38. Studies on the bioaccumulation of fenac by catfish (Ictalurus punctatus) were conducted in two 76-ℓ aquaria each containing 6 kg of sandy loam and 40 liters of dechlorinated tap water. The ^{14}C-fenac was added to the aquaria at a concentration of 2 ppm. After adding fenac,

the water was stirred and samples of water plus sediment were taken and counted for total ^{14}C. After a 30-day aging period, catfish were added to each aquarium. Prior to introducing the fish, aliquots of water were taken and counted for total radioactivity. The water samples were also extracted with diethyl ether following acidification and the ether extract was analyzed for fenac by gas chromatography using an electron-capture detector.

39. The fish and water samples were taken on 1, 3, 7, 14, and 21 days after introducing the fish into the aquaria. The fish were separated into head, viscera, and edible flesh portions. The amount of radioactivity in fish tissues was determined by combusting them in a Packard Tri-Carb Sample Oxidizer followed by liquid scintillation counting.

Bioaccumulation of fenac by Daphnia

40. The ability of fenac to accumulate in Daphnia magna was examined because significant accumulation in invertebrates might indicate a potential for bioaccumulation of fenac through trophic levels, leading to unacceptable concentrations in fish. Several hundred adult Daphnia were placed in one liter of artificial daphnid medium containing 2 ppm ^{14}C-fenac (specific activity = 3.9 μ Ci/mg). At periodic intervals, Daphnia were collected by transferring a portion of the exposure solution to a separatory funnel, allowing the Daphnia to settle to the bottom, and then drawing off the concentrated Daphnia. The daphnids were collected by filtration on pre-weighed 0.45-μ Millipore filters and washed with two 5-ml portions of water (the second wash contained no ^{14}C). The filters were weighed after air-drying and the Daphnia transferred to a glass homogenizing mortar by scraping them off the filter. This was necessary because some of the ^{14}C in the aqueous media adsorbed to the filters. The daphnids were ground with a Teflon pestle in a scintillation cocktail containing Triton X-100.

The cocktail was transferred to a scintillation vial. The mortar was rinsed with scintillation cocktail after which the cocktail was also added to the vial.

RESULTS AND DISCUSSION

Octanol-Water Partition Coefficient

41. The octanol-water partition coefficient (ratio of the equilibrium concentration of the chemical between octanol and water) has been used to define the lipohilic character of organic compounds. It has been shown that there is a linear relationship between octanol-water partition of a chemical and its ability to bioconcentrate in fish (9). Therefore, this partition coefficient can be used for predicting the bioconcentration, distribution, and persistence of chemicals in aquatic organisms. The octanol-water partition coefficient of fenac is given in Table 1.

Table 1
Partition Coefficient
(Fenac Concentration in Octanol/Fenac Concentration in Water)

Replicate	First Determination	Second Determination	Average
1	0.2096 (10:7)[1]	0.2063 (1:1)	
2	0.1787 (10:7)[2]	0.2020 (1:1)	0.206 ± 0.004

[1]Values in parentheses represent octanol:water volume ratios.
[2]Value rejected based on ± 2 standard deviation confidence limit.

The data show that the octanol-water partition coefficient of fenac is quite low. These results are consistent with the low bioaccumulation of fenac in aquatic organisms and with a low degree of sorption of fenac to sediment, as will be discussed later.

Adsorption of Fenac by Sediment

42. Since organic matter and clay content affect the sorption of

chemicals, these studies were conducted using four types of freshwater sediments varying in organic matter and clay content. The physical and chemical characteristics of the sediments (determined by the Department of Agronomy, Cornell University, Ithaca, N. Y.) are shown in Table 2.

Table 2

Physical and Chemical Properties of Sediments Used

Sediment Type	Particle-Size Distribution (% of Total)			% Organic Material
	Sand	Silt	Clay	
Organic Muck	2.3	18.2	79.5	54.0
Reduced Clay (Clay)	1.0	30.5	68.5	4.1
Oxidized Clay (Silty Clay)	2.0	67.0	32.8	1.6
Sandy Sediment	94.4	1.0	4.6	0.3

43. In preliminary studies, the rate of fenac adsorption to four types of sediments was determined at an initial fenac concentration of 2 ppm. Equilibrium studies at pH 6.5 revealed a fast initial uptake of fenac by the sediments. Maximum adsorption of the herbicide by all four sediments occurred within the first five hours (Figure 1). Subsequently, there was little or no change in the amount of fenac adsorbed. Therefore, all determinations were done after 24 hours of exposure.

44. An adsorption isotherm for fenac on the organic muck is shown in Figure 2. An increase in the aqueous phase fenac concentration resulted in a linear increase in the amount of fenac adsorbed by the sediment.

45. Table 3 shows the amount of fenac adsorbed by the sediment and the Kd (distribution coefficient) values. The low Kd values indicate that fenac is not significantly adsorbed by various sediments from water. As expected, the sandy sediment, because of its low organic matter and clay content, adsorbed the least amount of fenac in comparison to the other

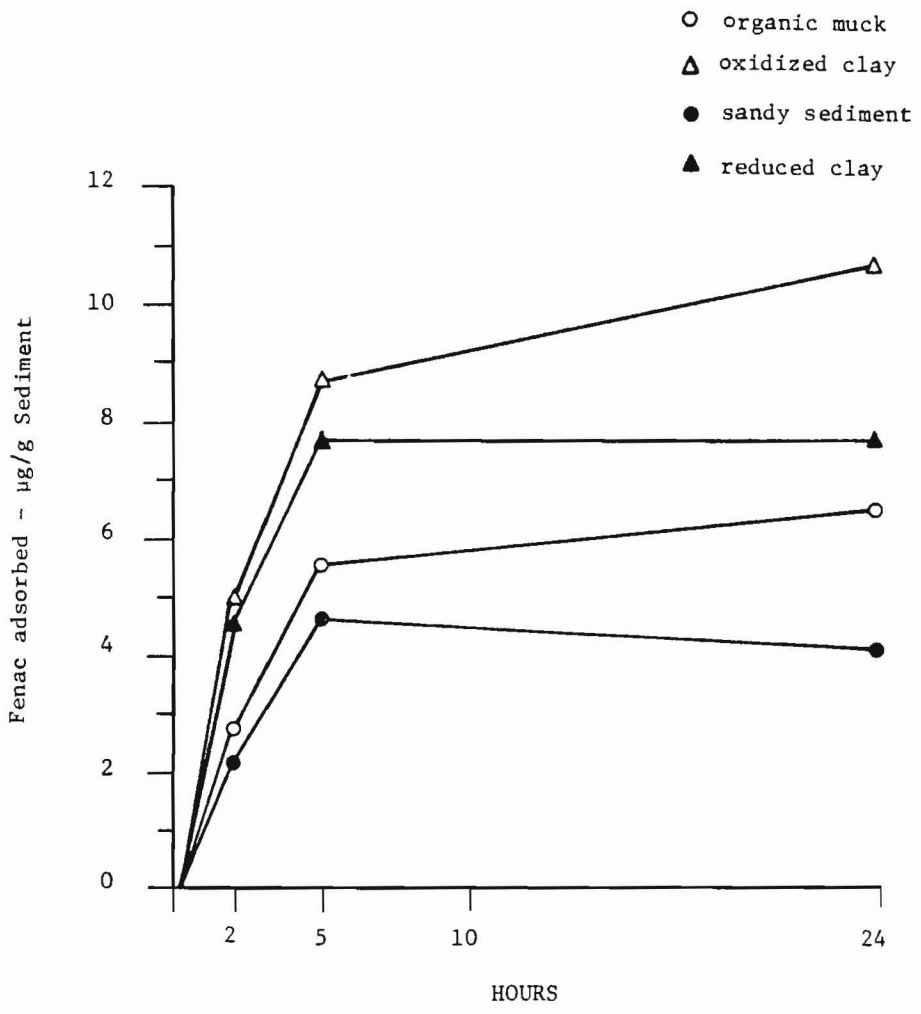

Figure 1. Adsorption of fenac to sediment

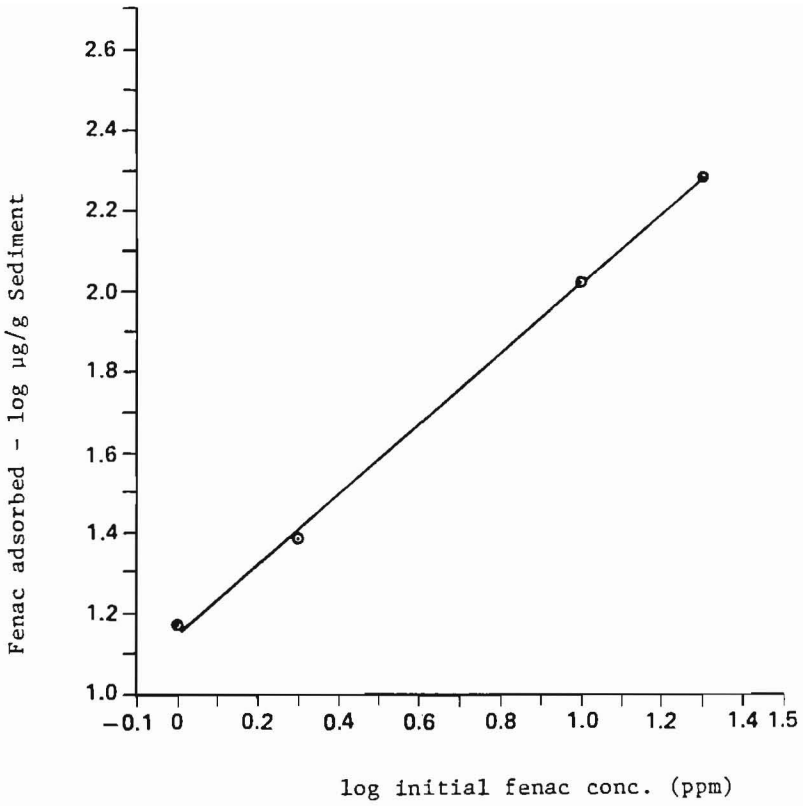

Figure 2. Adsorption isotherm for fenac in organic muck sediment at pH 4.5

sediments. A low adsorption of fenac by the sediments may be explained by the fact that at pH 6.5 fenac was predominately present as an anionic species, which is adsorbed considerably less than the undissociated species.

Table 3
Sorption of Fenac by Four Types of Sediment

Sediment	Fenac Adsorbed- µg/g Sediment	Kd
Organic Muck	6.3	3.3
Oxidized Clay	11.4	6.0
Reduced Clay	7.6	3.9
Sandy Sediment	3.9	2.0

46. The effect of pH on the sorption of fenac by the sediment was also examined. With a change in pH of the solution, the relative proportion of the anionic and undissociated fenac species will change, which may affect the sorption of the herbicide by the sediment. In these experiments, 0.5 g sediment and 50 ml of a 2 ppm solution of ^{14}C-fenac in the appropriate buffer (0.5 M acetate, pH 4:0.5 M borate, pH 9) were added to Erlenmeyer flasks and the sorption of the herbicide was determined.

47. Equilibrium studies at pH 4 showed a rapid initial uptake of fenac by the sediments with slower adsorption by the reduced clay continuing for 24 hours. Equilibrium studies at pH 9 showed a similar behavior (Figure 3). Table 4 shows the amount of fenac adsorbed by the four sediments at various pH's and the Kd values at 24 hours posttreatment. The results show that fenac is not adsorbed at pH 6.5 and pH 9 in significant amounts. At pH 4, which approximates the pK (pH at which fenac is half-ionized) of fenac (pK $\sim$ 3.7) (10), adsorption was considerably increased for all except the sandy sediment. However, even at pH 4, the capacity of the sediments to adsorb fenac is considered to be low.

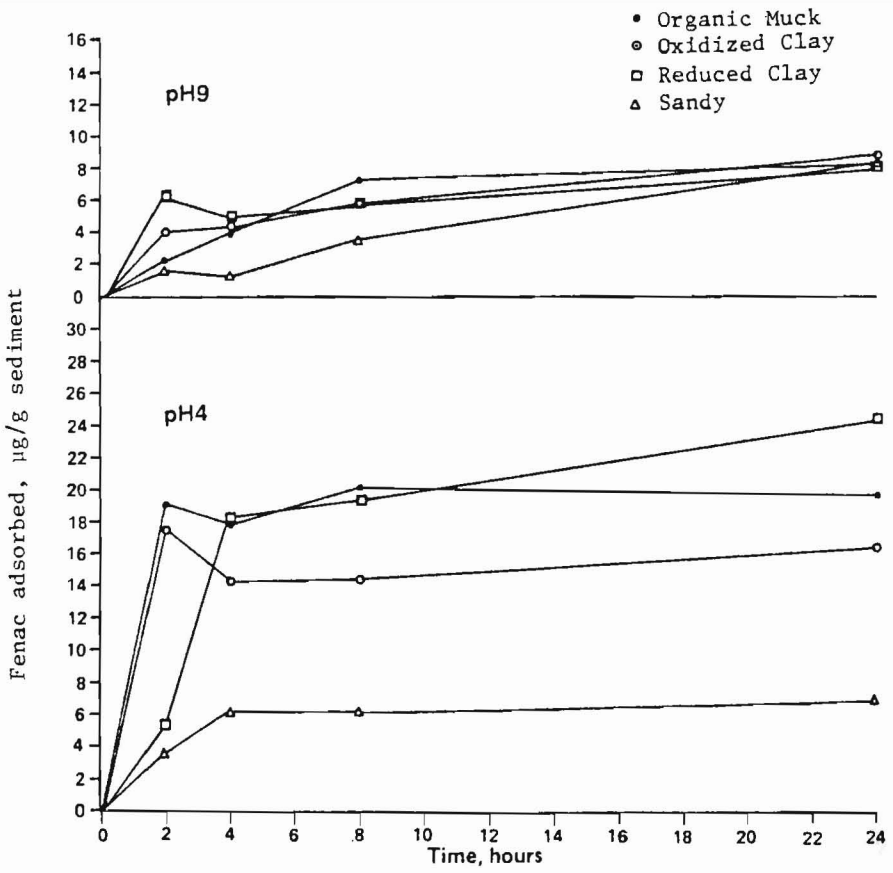

Figure 3. Effect of pH on the adsorption of fenac to sediment

Table 4

Effect of pH on Sorption of Fenac by Sediment

Sediment	pH 4 Fenac Adsorbed µg/g Sediment	pH 4 Kd	pH 6.5 Fenac Adsorbed µg/g Sediment	pH 6.5 Kd	pH 9 Fenac Adsorbed µg/g Sediment	pH 9 Kd
Organic Muck	19.7	12.2	6.1	3.31	8.5	4.42
Oxidized Clay	16.4	10.1	11.4	6.03	8.9	4.64
Reduced Clay	24.8	15.5	7.6	3.95	8.0	4.18
Sandy Sediment	6.9	4.68	3.9	2.01	8.3	4.35

48. The findings show that fenac sorption by different sediments is an important sink for the herbicide in the aquatic environment.

49. The desorption of fenac from the sediment was not investigated due to the low adsorption of the herbicide.

Hydrolysis of Fenac

50. The analysis of aqueous fenac solutions at pH 5.0, 7.0, and 9.0, following 3 and 6 weeks of incubation at 10° and 25°C, respectively, did not show any loss of the herbicide (Table 5). This suggests that fenac did not undergo hydrolysis during this period.

Table 5

Hydrolysis of Fenac

Temp.	25°C			10°C		
	pH 5.0	pH 7.0	pH 9.0	pH 5.0	pH 7.0	pH 9.0
Day	Fenac Concentration-ppm					
0	2.52	2.46	2.54	2.52	2.46	2.54
21	2.16	2.07	2.19	2.50	2.13	2.22
42	2.31	2.23	2.45	2.42	2.35	2.62

Photodegradation of Fenac in Aqueous Solution

51. No loss of fenac was observed following 36 hours of irradiation of a 2 ppm solution of the herbicide in distilled water at > 280 nm in a photochemical reactor. These findings suggest that the herbicide is likely to be resistant to degradation by sunlight.

Quantum yield of fenac*

52. The disappearance quantum yield ($\emptyset$) of a compound is a quantitative measure of its photodegradation rate. Values of $\emptyset$ normally range between 0 and 1, where $\emptyset = 0$ implies µg photolysis and $\emptyset = 1$ indicates that every photon absorbed leads to reaction. On occasions, $\emptyset$ can exceed unity, e.g. when photoinitiated chain reactions occur. The quantum yield for fenac was determined in 1:1 methanol:water (buffer) solutions at a wavelength of 254 nm. Ideally, the quantum yield should be measured in purely aqueous solutions at wavelengths corresponding to those found in sunlight i.e., > 290 nm. However, the limited solubility of fenac in water and its very weak absorption at 290 nm and above makes the measurement impractical under these conditions.

53. Quantum yields were measured at 254 nm in a Rayonet merry-go-round apparatus utilizing 1,3-dimethyluracil (DMU) in water as an actinometer (reference compound with known quantum yield). Solutions of fenac and DMU were optically matched at 254 nm, photolyzed in matched quartz tubes, and sampled periodically. The fenac solutions were quantified by HPLC, whereas the DMU samples were analyzed by UV spectrophotometry. The results listed in Table 6 indicate that the quantum yield of fenac is very low. Although these results were obtained in 1:1 methanol:water with a light wavelength of 254 nm, they are expected to be environmentally relevant since (a) the

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*Measurements made by Dr. Sujit Banerjee.

spectrum of fenac in water and 1:1 methanol:water should be very similar, and (b) the difference in wavelengths (254 nm as compared to > 290 nm in sunlight) should not greatly affect the quantum yield. Hence, direct photolysis is an unlikely pathway for the environmental degradation of fenac.

Table 6

Disappearance Quantum Yield of Fenac

	Minutes of Irradn.	DMU ($\times 10^{6}$ M)	Fenac ($\times 10^{4}$ M)	$\phi \times 10^{4}$	Solvent
Expt. 1	0	14.7	8.96		1:1
	3	11.4	-		methanol:
	7	8.28	-		water
	11	5.17	-		
	20	1.38	7.30		
	40	-	5.36	4.14	
Expt. 2	0	13.6	8.53		1:1
	3	11.0	"		methanol:
	5	9.80	"		borate
	6	8.39	"		buffer
	9	6.55	"		(pH=9.16)
	10	-	7.15		
	12	4.83	"		
	15	2.87	"		
	20	-	6.09		
	30	-	5.60		
	40	-	4.72	5.70	

Photodegradation of fenac in the presence of photosensitizers

54. It has been reported that the rate of photolysis of certain pesticides is considerably enhanced in the presence of naturally occuring photosensitizers (12, 13, 21). Since natural waters are known to contain photosensitizers, the photolysis of fenac dissolved in lake water was examined. Water samples collected from Oneida Lake and Jamesville Reservoir were used in these studies. The lake water was filtered through Whatman #1 paper prior to photolysis studies. A 2-ppm solution of fenac in lake water

was irradiated at 300 nm in a photochemical reactor. No degradation of herbicide was detected in the water samples from Jamesville Reservoir and Oneida Lake following 36 hours and 5 days of irradiation, respectively.

55. To investigate the possibility that natural photosensitizers such as chlorophyll may enhance photodegradation of fenac in natural waters, the photodegradation of the herbicide in water containing agal cells was examined. Fenac (2 ppm) was added to samples of lake water containing a high population of algae. The herbicide solution was placed on the roof top on a sunny day in mid-July and analyzed following 24 and 48 hours of exposure to sunlight. No loss of fenac was observed during the experimental period, suggesting that the algal pigments did not enhance photodegradation of the herbicide.

56. It is possible that the samples of lake water used in these studies did not contain sufficient amounts of photosensitizers. Therefore, studies were conducted to determine if fenac is degraded in the presence of known photosensitizers such as riboflavin phosphate (FMN). A 2-ppm solution of fenac in distilled water was irradiated in the presence of 1.0 and 0.1 g FMN/liter. The herbicide was observed to readily photodegrade in the presence of FMN; after 24 hours of irradiation, more than 75 percent of the herbicide had been lost. It was noticed in these studies that FMN was photodegraded at a significantly higher rate than fenac. While this presents some problems in the laboratory, it does not detract from the importance of photosensitized degradation of fenac in the environment, since photosensitizers would most likely be replenished from the decomposing organic matter.

57. The photodegradation of fenac was also examined in the presence of other photosensitizers such as methylene blue, benzophenone, and acetone. Neither benzophenone nor acetone at concentrations ranging from 1-100 ppm had any effect on photodegradation of fenac within 24 hours. However,

methylene blue at 1 ppm enhanced the photodegradation of the herbicide. The rate of photolysis of fenac in the presence of methylene blue was lower than that noticed with FMN. Methylene blue also underwent photodegradation in the presence of air. However, unlike FMN, it did not photodegrade in degassed, argon-saturated water.

58. The photodegradation of fenac was examined in the presence of another naturally occurring photosensitizer, i.e., a commercially available "humic acid" (Aldrich Chemical Company). This substance is a derivative of decayed plant material and its electronic absorption spectrum is very similar to that of materials dissolved in natural water. The "humic acid" (1 mg/ℓ) was added to degassed distilled water containing 4 ppm of fenac. The solution was then photolyzed in a photochemical reactor (Hanovia medium pressure mercury lamp with pyrex filter) for six hours with oxygen-free argon continuously bubbled through the solution. Aliquots of the photolyzed solution were removed at two-hour intervals and analyzed for fenac by HPLC. The results presented in Table 7 show that more than 50% of the herbicide was photodegraded within six hours. These results show that fenac can be photodegraded in an aqueous solution in the presence of materials dissolved in natural waters.

59. We also examined the photodegradation of fenac in aerated distilled water containing humic acid. Under these conditions, no degradation of the herbicide was noticed within 24 hours of irradiation. The probable explanation for the lack of degradation in the presence of air is the photodestruction of the sensitizer (as indicated by bleaching) under these conditions. However, under natural conditions where photosensitizing materials would most likely be replenished from the decomposing organic matter, photosensitized degradation may play a significant role in the dissipation of fenac.

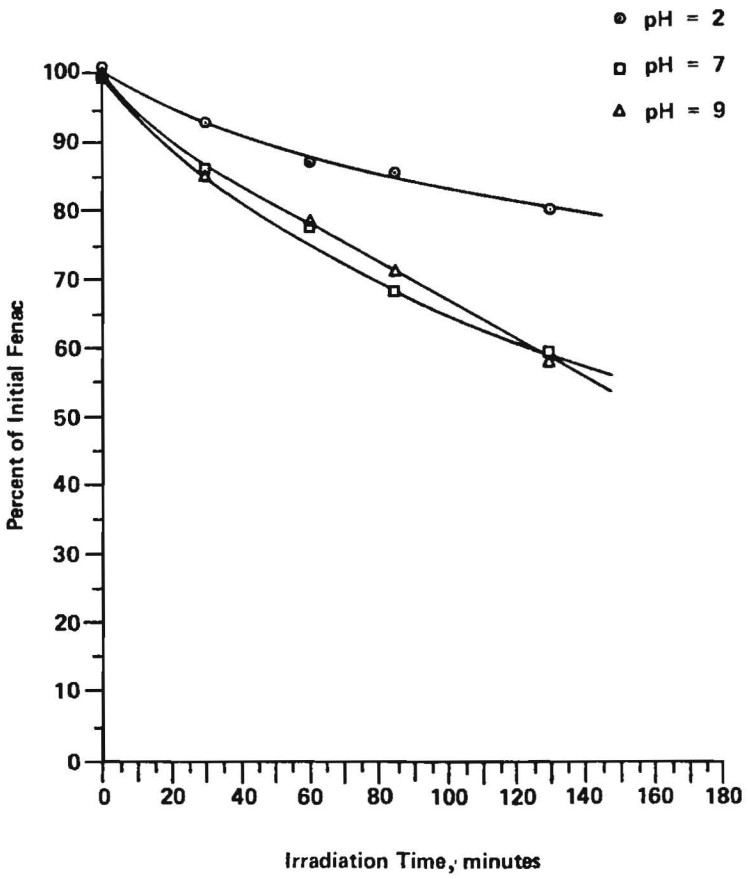


Figure 4. The pH dependence of fenac photodegradation at 254 nm

Table 7

Photodegradation of Fenac (4 ppm) in the Presence of Humic Acid

Irradiation Time (Hrs.)	% Fenac Remaining
0	100
2	93.9
4	79.1
6	47.7

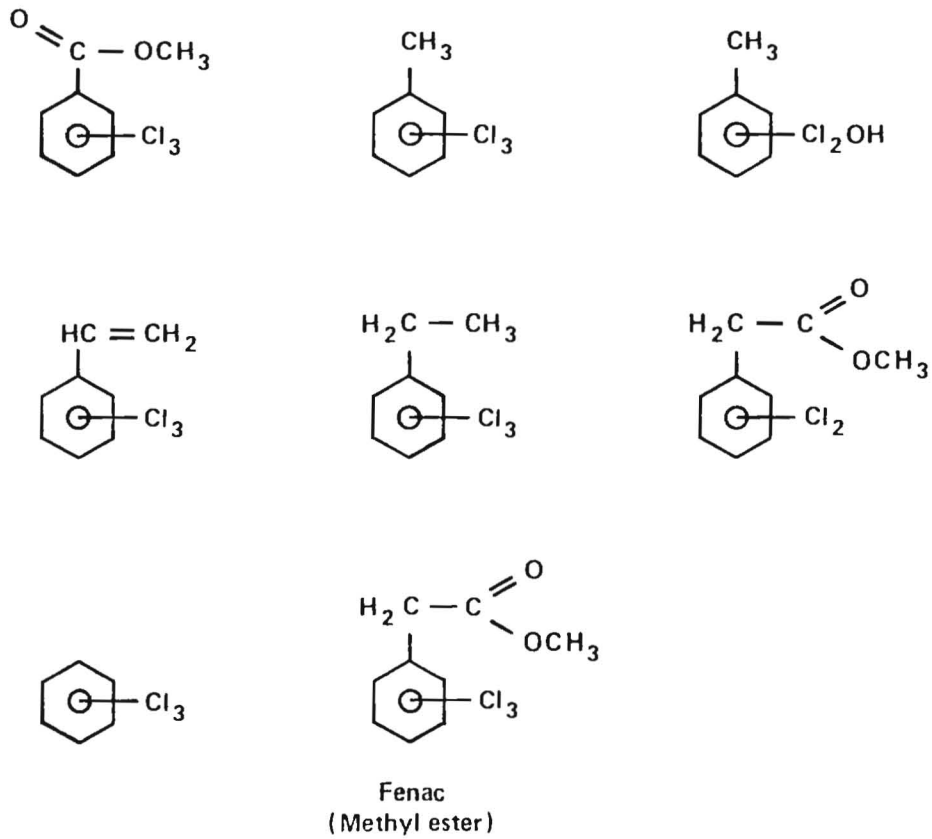
60. These findings indicate that although fenac is not readily photodegraded in distilled water, it will be degraded in natural waters by the action of sunlight due to the presence of naturally occurring photosensitizers.

Effect of pH on the photodegradation of fenac

61. A number of experiments were conducted to determine the effect of pH on the rate of fenac photodegradation. Aliquots of 10 ml of an aqueous solution of fenac (40 ppm) buffered to pH values of 2, 7, and 9 were irradiated in a Rayonet miniphotochemical reactor equipped with a 2.2-watt, 254-nm wavelength lamp. The results, illustrated in Figure 4, clearly demonstrate that photodegradation is retarded in an acidic medium, but is relatively insensitive to pH in neutral or alkaline solutions.

Products of fenac photodegradation

62. On the basis of GC-mass spectral analysis, the compounds shown in Figure 5 were tentatively identified in the photolysate following irradiation of an aqueous solution of fenac for 24 hours in the presence of FMN. Photolysis of the fenac solution at 300 nm in the presence of FMN resulted in a complex mixture of degradation products (Figures A-F of Appendix A). Some of the products included oxidized and reduced products which resulted from the replacement of ring chlorines of fenac isomers by hydroxyl groups or hydrogen. The mechanisms by which other photodegradation products were generated are not known.



Fenac
(Methyl ester)

Figure 5. Products of fenac photodegradation

63. These findings show that although light (300 nm) caused the transformation of fenac in the presence of certain photosensitizers, it did not result in an extensive degradation of the molecule. Most of the degradation products included compounds in which only the side chain had been altered. These results suggest that although photodegradation may cause extensive dissipation of fenac in natural waters containing photosensitizers, the process may result in the formation of degradation products which may be relatively persistent due to the presence of an intact aromatic nucleus with two or more chlorines.

Biodegradation of Fenac in Lake Water and Sediment

64. The concentrations of fenac in water samples taken at various times after treatment are shown in Figure 6. The dissipation of herbicide from the water in various aquaria followed a similar pattern. During the first 6 weeks, extremely large variations in fenac concentration in water were noticed. Subsequently, the fenac levels in water showed considerably less fluctuation. After the fenac levels in water had somewhat stabilized, a gradual decrease in the concentration of the herbicide was observed. These findings suggest that fenac is slowly degraded by aquatic microorganisms. The initial large decrease of fenac was believed to be due to adsorption by the sediment. Calculations based on the data concerning adsorption of fenac to these sediments indicate that the sediments used in the aquaria are cabable of adsorbing from 23.4-68.4 mg of fenac, depending on the type of sediment. Since there was only 12 mg of fenac present in the aquaria at zero time, the possibility exists that a significant fraction of the herbicide added to the water was adsorbed by the sediment.

65. Experiments were initiated to study biodegradation of fenac in natural waters under more controlled conditions in an attempt to explain

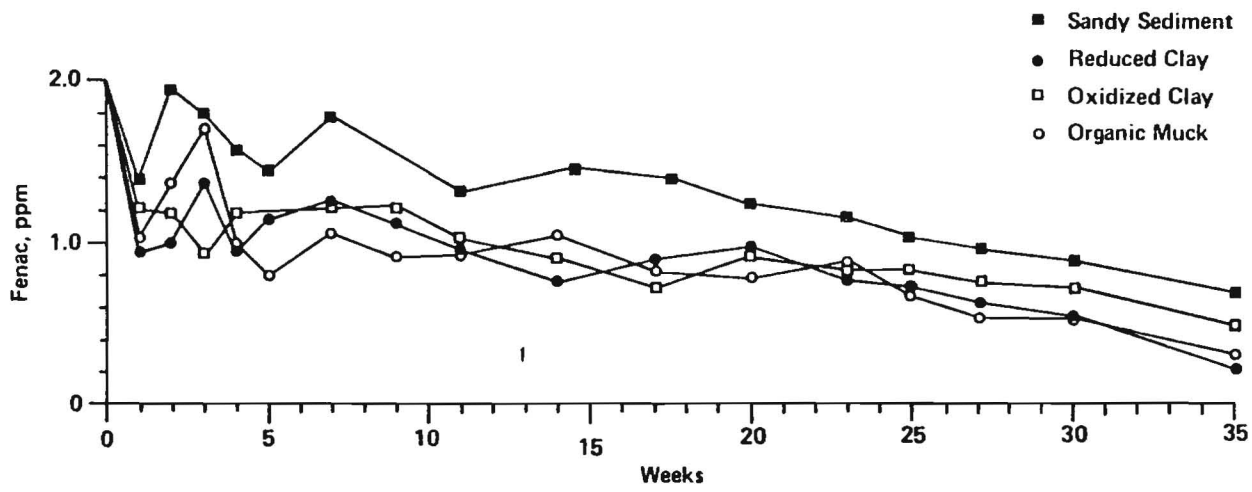


Figure 6. Fenac residues in water in aquaria treated with 2 ppm of the herbicide

fluctuations noticed in the aquarium studies. Two 2.8-ℓ Fernbach flasks containing 1 ℓ of lake water and a 5-cm layer of sediment were placed in a constant-temperature chamber. After the sediment had settled, ^{14}C-labelled fenac at a concentration of 2 ppm was introduced slowly so as not to disturb the sediment. Periodically, aliquots of water were taken and analyzed for fenac. No radioactivity was detected in the ether extract at pH 9 (fraction 1) and in the aqueous phase after extraction with ether at pH 2 (fraction 3). Essentially all of the radioactivity in the water samples was associated with the ether extract at pH 2 (fraction 2). Gas-chromatographic analysis of this fraction indicated only the presence of fenac. During the initial time period, the same pattern was observed in the levels of fenac in flasks (Figure 7) as in the larger aquaria (Figure 6). After the initial equilibrium phase, the fenac concentrations slowly decreased by about 50 percent (1 ppm), as they did in the larger aquaria. The absence of degradation products in water suggests that the decrease in fenac concentrations in the water was primarily due to the adsorption to the sediments.

66. The resistance of fenac to microbial degradation was further confirmed in an experiment dealing with the uptake of fenac by catfish. In these studies, fenac was added to aquaria containing sandy loam soil, water, and catfish. The water was analyzed for fenac at various intervals after adding the herbicide to the aquaria. The results showed that the concentration of fenac in the water did not change appreciably during the 4-week period after the fish were introduced into the aquaria (refer to studies on the Bioaccumulation of Fenac by Catfish).

Biodegradation of Fenac under Anaerobic Conditions

67. It has been reported that DDT and certain other chlorinated hydrocarbon pesticides are transformed by microorganisms more rapidly under

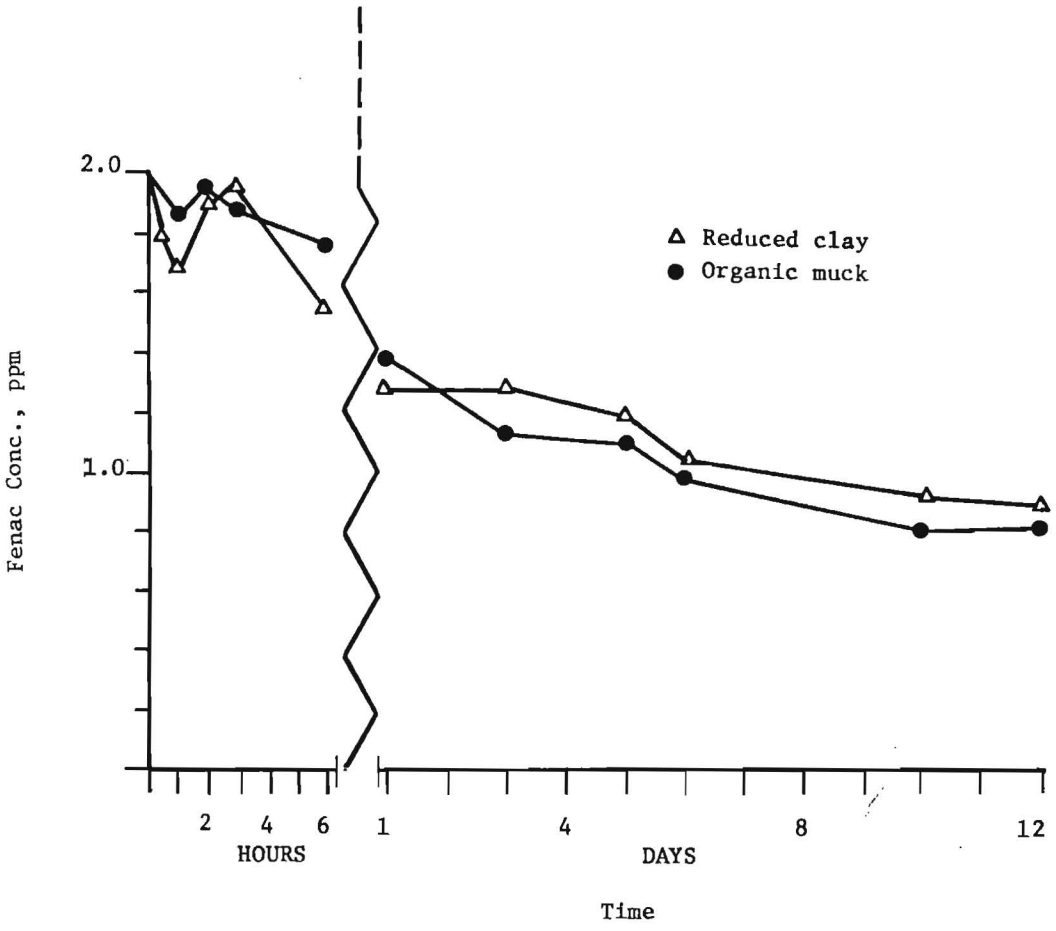


Figure 7. Fenac residues in water in 2.8-ℓ flasks containing water and sediment

anaerobic conditions than under aerobic conditions (10, 11). It is conceivable that fenac, like DDT, may be dechlorinated more readily under anaerobic conditions. The loss of chlorine from the molecule may make it more susceptible to microbial attack.

68. Studies on the biodegradation of fenac under anaerobic conditions were performed in aquaria (containing water, sandy loam soil, and fenac) which had been previously used for studying the bioaccumulation of ^{14}C-fenac by catfish (for experimental details refer to Bioaccumulation of Fenac by Catfish). These aquaria were used to assess the biodegradation of herbicide under anaerobic conditions after the bioaccumulation studies were terminated. To create microaerophilic or partially anaerobic condiditons, one of the aquaria was bubbled with nitrogen for two weeks and then sealed using a glass plate and sealing tape. Water samples were taken from each aquarium at 3-week intervals and analyzed for fenac by GLC or HPLC. The water level was maintained by adding filtered tap water 2-3 days prior to sampling (to allow time for the sediment to settle before removing water samples).

69. The results did not show degradation of the fenac under anaerobic conditions (Table 8). The fluctuations in the herbicide concentration at various times were probably due to not maintaining the volume of water in the aquaria precisely ($\pm$ 2 liters for a total volume of 40 liters).

70. These findings regarding the persistence of fenac are in agreement with those of other workers. Hodgson, Frank, and Comes (4) studied the persistence of fenac in the water and hydrosoil of two ponds treated with the herbicide at concentrations of 1-1.56 ppm. The herbicide residues could be detected in both the water (0.07-0.38 ppm) and sediment (0.08-0.26 ppm) after 160 days of

treatment. Sheets, Smith, and Kaufman (15) reported that fenac is slowly degraded as a result of microbial action in different soils under greenhouse conditions.

Table 8

Degradation of Fenac in Water and Sediment Under Anaerobic Conditions

Weeks after Anaerobic Treatment	Fenac Concentration in Water - ppm
0	1.47
4	1.47
6	1.46
10	1.21
13	1.34
18	1.55
22	1.45
26	

Effect of Fenac on Microbial Activities in Sediment

71. Microorganisms play an important role in aquatic ecology, particularly in producing and recycling nutrients for plants and higher organisms. An adverse effect of fenac on aquatic microorganisms may lead to significant changes in the ecosystem. The effect of fenac on several physiological functions of microbial populations in water and sediment was examined. These included tests for respiration, nitrification, and cellulose, starch, and protein degradation.

Respiration

72. Results presented in Table 9 and Figure 8 show that fenac concentrations up to 10 ppm had no effect on oxygen consumption or CO_2 evolution by microorganisms in the sediment.

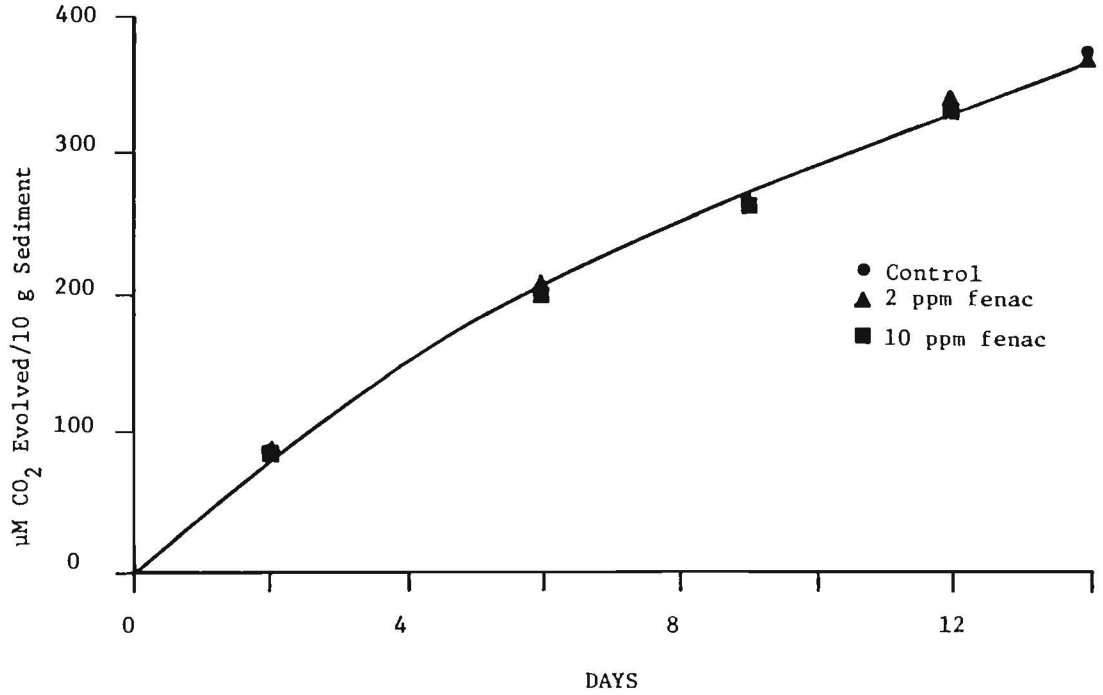


Figure 8. Effect of fenac on CO_2 evolution in sediment

Table 9

Effect of Fenac on Oxygen Consumption by Microorganisms in Lake Sediment

Time	μℓ O_2 Consumed/g Sediment		
	Control	2 ppm Fenac	10 ppm Fenac
0 - 18 hours	64	66	62
48 - 56 hours	54	58	56
96 - 104 hours	58	56	55
144 - 152 hours	53	55	51

Nitrification

73. The results given in Table 10 show that fenac concentrations up to 10 ppm had no effect on nitrification in sediment.

Table 10

Effect of Fenac on Nitrification in Sediment

Treatment	Time (days)	Total Nitrate Detected (μg)				
		0	7	14	21	28
Control without ammonium sulfate		150	-	-	-	330
Control		150	150	580	850	1320
2 ppm fenac		150	150	460	760	1320
10 ppm fenac		150	210	590	730	1280

Cellulose degradation

74. The results depicted in Figure 9 show that the rate of mineralization of cellulose carbon in sediment containing 2 ppm fenac was the same as that of the control. At the end of 32 days, 52 percent of the carbon was mineralized in each. In sediment containing 10 ppm fenac, about 60 percent of the ^{14}C-cellulose was degraded to $^{14}CO_2$ after 32 days. These findings suggest that fenac has no effect on cellulose degradation in the sediment at concentrations up to 10 ppm.

Starch degradation

75. The results depicted in Figure 10 show that at the end of 25 days, 77 percent of starch was mineralized in the control flasks, 78 percent in flasks containing 2 ppm fenac, and 78 percent in flasks containing 10 ppm fenac. The results show that fenac up to 10 ppm did not have any effect on degradation of starch in the sediment.

Protein degradation

76. The data presented in Table 11 and Figure 11 show that fenac up to 10 ppm had no effect on the amount of CO_2 produced or on the amount of nitrate produced as a result of the addition of casein to the sediment.

Table 11

Effect of Fenac on Nitrate Production from Casein Degradation in Sediment

	µg NO_3^-/Flask			
Time	Unamended Control	Control w/Casein	2 ppm Fenac w/Casein	10 ppm Fenac w/Casein
0	25	-	-	-
2 weeks	40	250	240	250

77. Based on the results of the authors' studies concerning the effect of fenac on a variety of microbial processes in the sediment, it appears that fenac has no adverse effect on the activity of aquatic microorganisms at levels considerably higher than field application rates.

Effect and Biodegradation of Fenac in Activated Sludge

78. The effects of fenac on wastewater treatment processes were assessed in laboratory studies with activated sludge. These studies were designed to determine the degradation rate of fenac and to assess the effects of the herbicide on microbial respiration as measured by CO_2 evolution.

79. Essentially all of the radioactivity in acidified aqueous solutions was extracted into ether at all sampling times. Thin-layer chromatography of

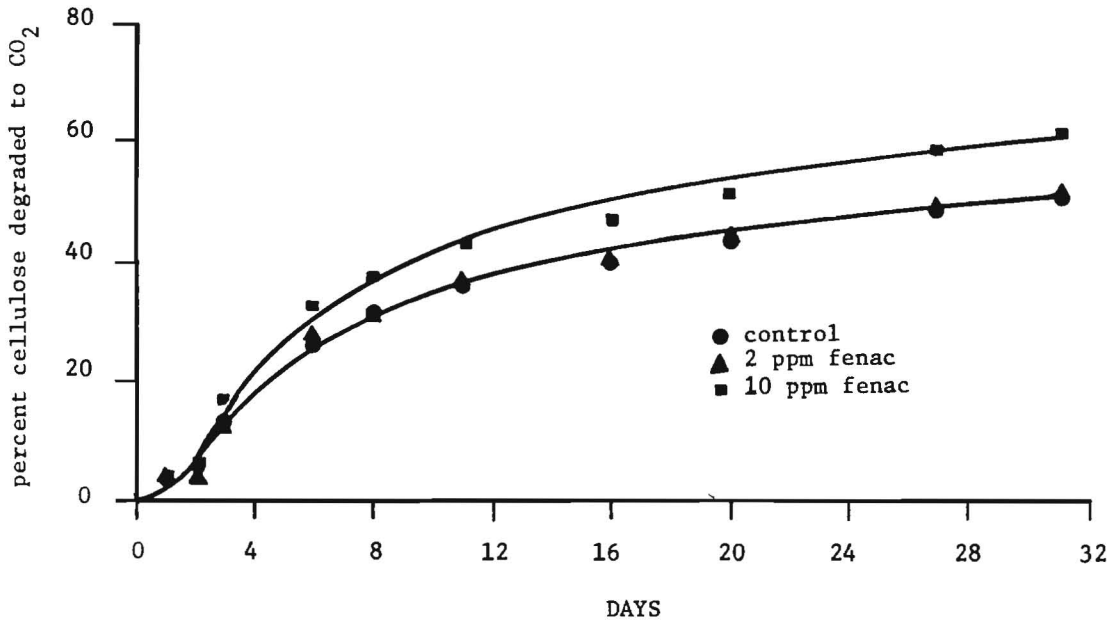


Figure 9. Effect of fenac on microbial degradation of cellulose in sediment

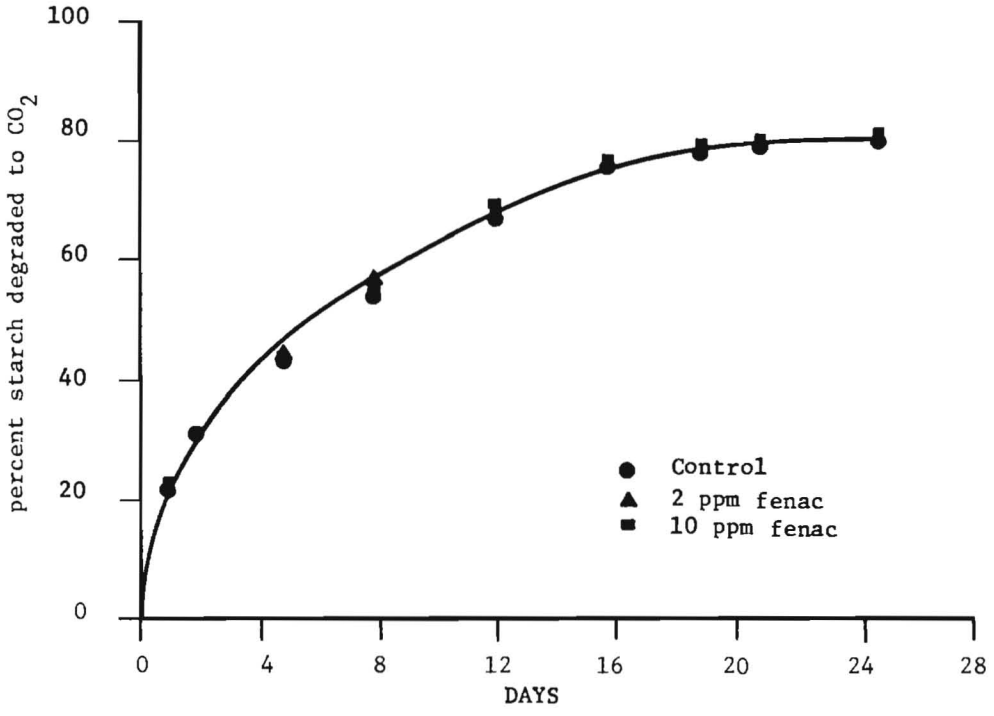


Figure 10. Effect of fenac on microbial degradation of starch in sediment

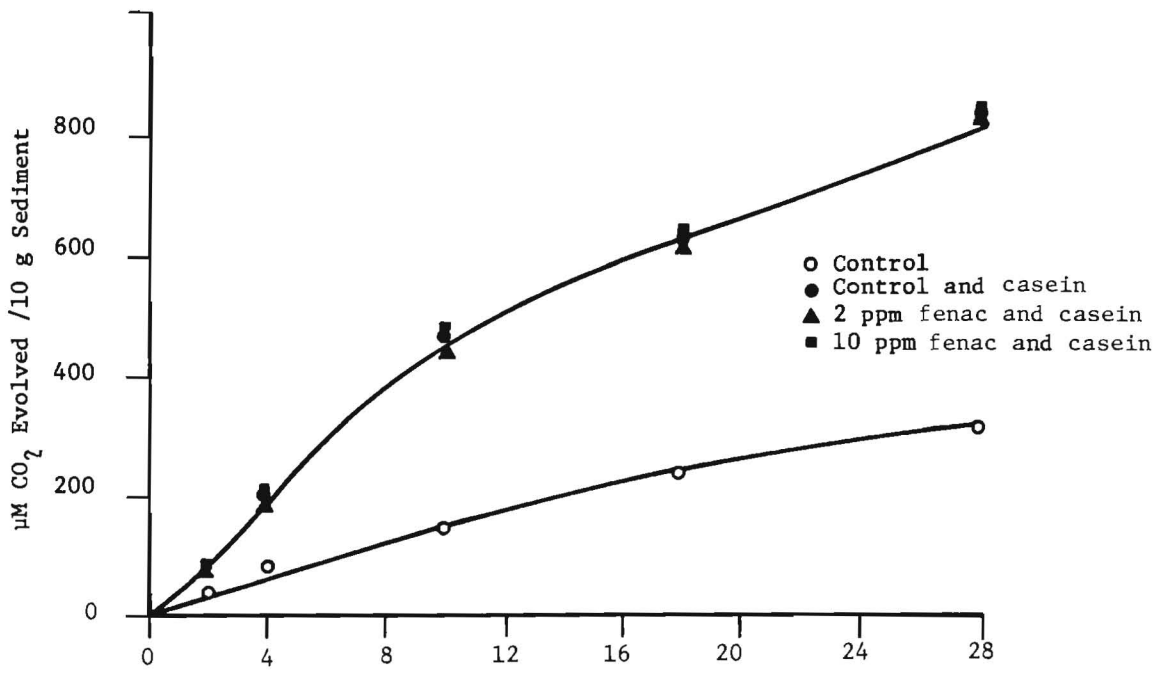


Figure 11. Effect of fenac on microbial degradation of protein in sediment

the ether extracts from samples obtained on the 12th and 16th days indicated the presence of only one radioactive peak, which co-chromatographed with authentic fenac. HPLC analysis also showed that only fenac was present in the extracts. No $^{14}CO_2$ was evolved from the unit during the experimental period. The results suggest that fenac was not degraded by the micro-organisms in the activated sludge during the test period.

80. Table 12 shows the material balance of fenac added to the sludge unit. Since negligible degradation of the herbicide was observed, the amounts of fenac present in the supernatant are based on the amount of total ^{14}C assayed by liquid scintillation counting. Over the 16-day treatment period, about 79 percent of the added herbicide was recovered unaltered in the supernatant and about 21 percent was associated with the settled sludge. Analysis of the settled sludge at the termination of the experiment showed that 41 percent of the radioactivity was sorbed to the sludge solids and the remainder was in aqueous solution.

81. The total amount of carbon dioxide evolved from the treatment and untreated sludge units during the test period is shown in Table 13. The results show that fenac concentrations to about 78 ppm (concentrations in the sludge after the final treatment) had no toxic effect on the microbial activity in the activated sludge.

Table 12

Fate of Fenac in the Activated Sludge Unit

	mg Fenac			
Day	Added to 1500 ml (at the beginning of daily cycle)	Total present at the beginning of cycle* (Amount remaining in sludge plus added)	Measured in 1000 ml effluent	Measured in 500 ml settled sludge
1	0.15	--	--	--
2	0.3	0.3	0.3	0.1
3	0.6	0.7	0.4	0.3
4	1.2	1.5	0.9	0.7
5	1.5	2.2	1.3	0.8
6	3.0	3.8	1.8	1.8
7	4.5	6.3	3.7	2.3
8	6.0	8.3	4.9	4.7
9	7.5	12.2	6.3	4.9
10	9.0	13.9	8.9	5.7
11	10.5	16.2	10.4	5.7
12	12.0	17.7	10.9	8.0
13	13.5	21.5	11.7	9.8
14	15.0	24.8	12.9	10.1
15	30.0	40.1	24.2	18.4
16	60.0	78.4	39.9	36.9
Total mg Fenac	174.75		138.5	110.2

*Represents fenac measured in settled sludge from previous day (last column) plus fenac added with new sludge (second column).

Table 13

Effect of Fenac on CO_2 Evolution by Microorganisms in Activated Sludge

Day	Fenac Concentration at the beginning of cycle	mM CO_2 Evolved: Control Sludge	mM CO_2 Evolved: Fenac treated Sludge
1	0.1	5.6	5.7
2	0.3	5.8	5.6
3	0.7	5.9	6.0
4	1.5	6.0	6.1
5	2.2	6.2	6.4
6	3.8	6.5	6.2
7	6.3	6.7	6.3
8	8.3	5.8	5.8
9	12.2	7.2	7.0
10	13.9	6.0	6.2
11	16.2	8.2	8.3
12	17.7	6.7	7.2
13	21.5	7.3	7.0
14	24.8	7.0	6.8
15	40.1	7.9	7.4
16	78.4	7.6	7.8

Toxicity of Fenac to Fish

Fathead minnows

82. The results, listed in Table 14, indicate that fenac showed negilible toxicity to fish up to 40 ppm under the conditions of a 96-hr static bioassay. Through 96 hr, no abnormal behavior or symptomology was noted in the treated fish relative to controls. Dissolved oxygen content in 96 hr ranged from 6.4 to 7.7 ppm. Use of fenac concentrations higher than 40 ppm is not contemplated due to the necessity of using emulsifying agents or high pH ($\geq$ 9) to achieve such concentrations and it is unlikely that the herbicide

will be present at such high concentrations in the environment.

Table 14

Acute 96-hr Toxicity of Fenac to Fathead Minnows

Concentration (ppm)	% Morality*
0 (control)	0
40	10
30	20
20	10
10	10
5	0

* Data are the average of 10 fish per treatment level

Rainbow trout

83. The results on the toxicity of fenac to rainbow trout, listed in Table 15, show that fenac is without acute toxic effects under the experimental conditions, thereby confirming the results with fathead minnows. While toxic effects cannot be excluded for theoretical concentrations above 40 ppm, it is highly unlikely that such levels can be achieved in the environment due to limits of solubility of the herbicide.

Table 15

Acute 96-hr Toxicity of Fenac to Rainbow Trout

Fenac (ppm)	% Mortality
0	0
0.4	0
4.0	0
40.0	0

Effects of Fenac on Daphnia

84. Qualitative and quantitative changes in zooplankton populations of natural waters following treatment with various chemicals can drastically alter the ecological balance of the systems. Population decline of zooplankton, a major food source in the early life stages of most fish, can ultimately lead to a decline in populations of food and game fish species. The cladoceran Daphnia magna has become a model species for evaluating the toxicity of chemicals to zooplankton. In order to help predict the effects of fenac application in natural waters, the effect of fenac on Daphnia reproduction was examined, as well as its direct toxic effects.

Acute toxicity

85. The acute toxicity data were plotted as percent mortality versus toxicant concentration on probability/log paper, and mortality curve was fitted by hand for determining the EC_{50} (Figure 12).

The EC_{50} obtained in this study, 28 ppm, is considerably lower than the value of EC_{50} > 100 ppm reported by both Crosby and Tucker (2) and Sanders (14) at 28 and 48 hours, respectively. This might be due to the use of highly pure fenac in this study, giving a higher concentration of the active agent per weight of material in solution. For instance, Sanders (14) utilized a wettable powder fenac formulation in his studies. Also, as discussed below, fenac toxicity may require several days to occur.

86. An additional study was conducted to examine the toxicity of fenac within the range of 20 to 0.02 ppm, concentrating around the application rate of 2 ppm. The results are presented in Table 16.

Table 16

Acute 96-hr Toxicity of Fenac to Daphnia magna

Fenac (ppm)	% Mortality (corrected)* + Std. Dev.
20	17.1 ± (5.2)
10	9.7 ± (8.8)
5	10.2 ± (6.0)
3.2	0 ± (0)
2.6	5.0 ± (3.0)
2.0	2.2 ± (0.5)
1.4	0.5 ± (0.5)
0.8	2.7 ± (2.9)
0.2	0 ± (0)
0.02	0.5 ± (0.5)

* The data are the average of 3 experiments, each with 20 Daphnia per replicate.

87. These results indicate that fenac is not lethal to Daphnia at levels below 5 ppm over a 96-hour period, as also shown by the slope and extrapolated X-intercept of the dose-response curve (Figure 12). Doses of 5-20 ppm indicated only a very small toxic effect on Daphnia.

88. The acute 96-hour EC_{50} of fenac to Daphnia was found to be 29 ppm in a second experiment. Therefore, based on the two different determinations, the 96-hour EC_{50} of fenac to Daphnia is 28.5 ± 0.5 ppm.

Daphnia reproduction

89. Populations of Daphnia might decline in natural waters treated with fenac not only due to direct toxic effects, but also through more insidious means, particularly by reducing the reproductive potential of the Daphnia. To test the latter possibility, the effect of fenac on Daphnia reproduction was examined. Fenac appeared to have a definite long-term toxic effect on Daphnia at levels lower than the acute 96-hour EC_{50} (Table 17). However, the number of replicates per treatment were

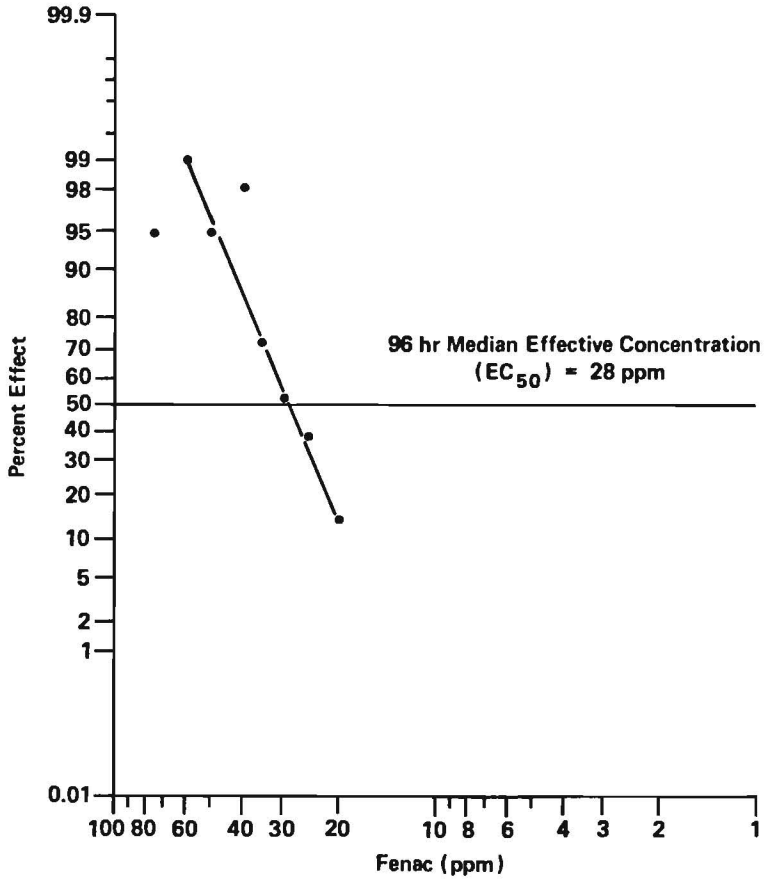


Figure 12. The 96-hour toxicity of fenac to Daphnia magna

rather low to permit a complete statistical treatment. The concentration response effect of fenac on total production of young Daphnia relative to controls is given in Figure 13; the graph shows a linear relationship between concentration and total number of young produced. At a fenac concentration of 9 ppm, reproduction is inhibited by 50 percent with an apparent no-effect level of 0.2-0.3 ppm. Analysis of mean brood size between controls and the treated animals showed no significant effect by fenac on this parameter at the 0.01 level of significance. Therefore, it appears that the major factor contributing to reduction of Daphnia productivity by fenac is the long-term toxicity of the chemical rather than interference with the reproductive physiology of the organisms. These results show that fenac will have a minimal (i.e. less than 20 percent reduction in first generation productivity) impact on Daphnia reproductive success at application levels of 2 ppm or less.

Table 17

Effect of Fenac on Daphnia magna Reproduction*

Fenac (ppm)	21 Day Adult Survival	Total Young Produced	Mean Brood Size
0.0	90%	407	9.9
0.5	70%	360	11.6
2.0	40%	323	11.1
5.0	40%	241	10.5
20.0	30%	155	11.1

* Values were obtained from treatment populations containing 10 animals each.

90. The above experiments showed that fenac had little or no effect on reproductive success of Daphnia magna at levels of 2 ppm or less. However, because of small initial sample size, the experiment was repeated in part with a larger sample size to increase the accuracy of statistical analysis.

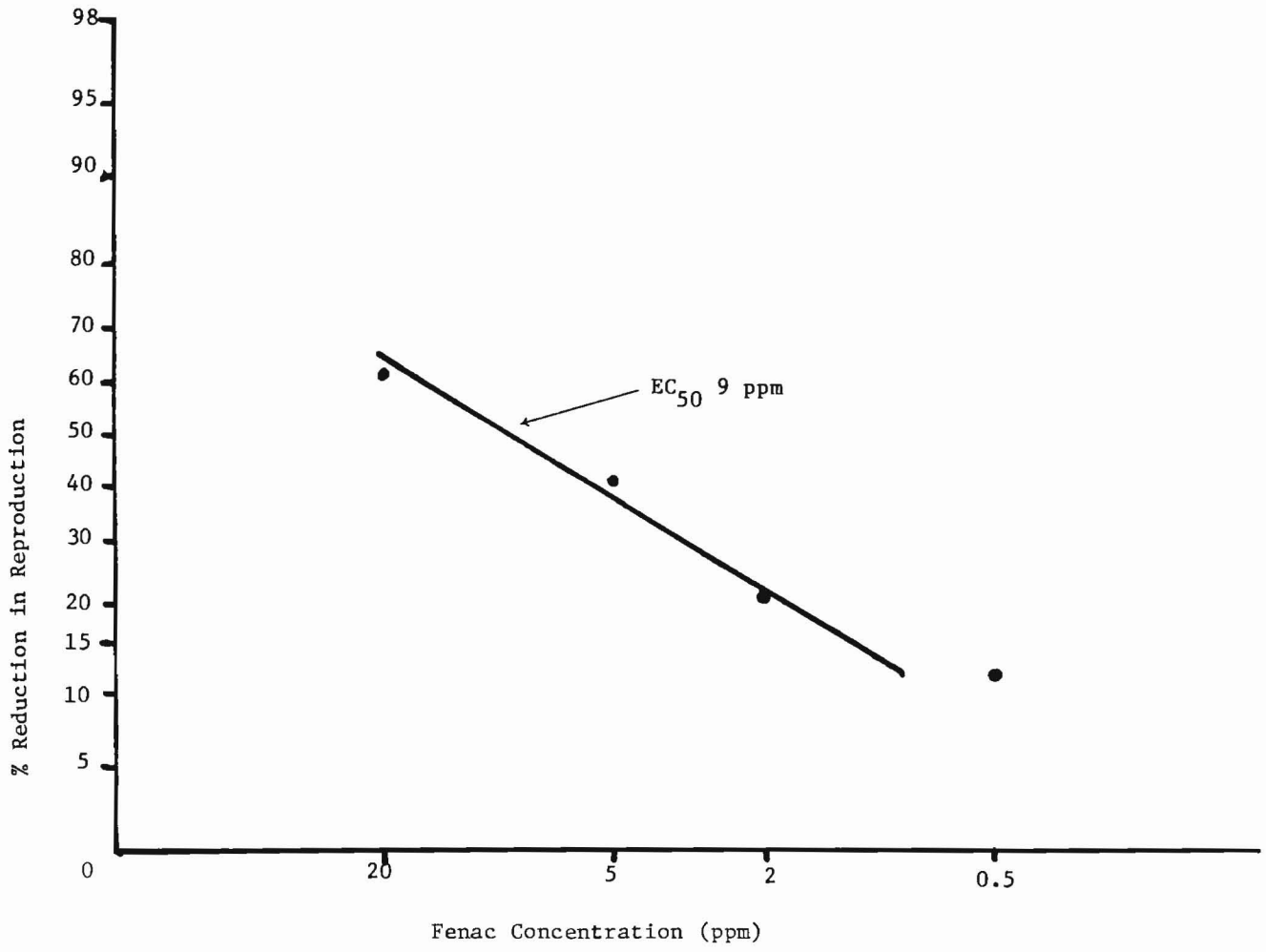


Figure 13. Effect of fenac on Daphnia reproduction

In these studies, the initial treatment and control populations each consisted of 15 Daphnia. The reproductive effect of fenac was studied only at the 2.0 ppm application level (Table 18). There was no significant effect of fenac on mean brood size or total brood number for Daphnia magna. These findings suggest that the application of 2 ppm fenac will have a minimal direct impact upon aquatic zooplankton.

Table 18

Effect of 2.0 Fenac on Daphnia magna Reproduction

Fenac (ppm)	Total Young Produced	Total Brood Number	Mean Brood Size
0.0	638*	71	8.99*
2.0	547	65	8.42

* Two values not significantly different at $p > 0.01$.

Bioaccumulation and Metabolism of ^{14}C-Fenac by Bluegill Sunfish

Static exposure studies

91. Preliminary experiments were conducted in which bluegills were exposed to ^{14}C-fenac for 5 days under static conditions. The results showed that fenac has a low bioaccumulation potential (Table 19). The head and viscera contained a majority of ^{14}C-residues detected in the fish.

Table 19

Residues of Radioactivity in Bluegills Exposed to Water Containing ^{14}C-Fenac

Exposure Time	^{14}C-Residue* (expressed as µg of fenac/g of tissue) Edible Flesh	Head & Viscera	Total Body
5 Days	0.37 (12%)	3.00 (88%)	1.61
	BCF = 0.185	BCF = 1.5	BCF = 1.3

* Represents average of seven fish
BCF = Bioconcentration factor

Flow-through exposure studies

92. Experiment #1. The studies on the uptake and elimination of ^{14}C-fenac by fish exposed to 2 ppm of the herbicide are shown in Figure 14. The levels of ^{14}C appeared to reach equilibrium levels in the edible portions of the fish between 6 and 9 days of exposure, while the levels of nonedible portions equilibrated between 9 and 13.5 days. The bioconcentration factor (BCF) (concentration of ^{14}C-fenac equivalents in fish/concentrations of ^{14}C-fenac in water) was found to be 0.7 for edible tissue (average of 6-15.5 day values) and 2.8 for nonedible tissue (average of 9-15.5 day values). These findings show that fenac has a low potential for bioaccumulation in fish.

93. The rate of elimination of ^{14}C-residues was determined by plotting log % retention vs. time (Figure 15). The rate of elimination from edible tissue is first-order with time, with an elimination rate constant, k, of 0.050 and a half-life of 11.5 days. The elimination of ^{14}C-residue from nonedible tissue appears to be biphasic. The initial fast phase of elimination (0-7 days of depuration) showed $k = 0.120\ day^{-1}$ and half-life = 16.4 days. These results show that fenac is continually eliminated from the fish after removal from sources of contamination, and should not present a significant residue problem.

94. Experiment #2. An additional study on the bioaccumulation of ^{14}C-fenac was performed. The results of this study are shown in Table 20. At equilibrium (120-240 hours of exposure), BCF's of 8.3 in edible tissue and 10.1 in nonedible tissue were achieved. These values are somewhat higher than the previously reported studies but still do not suggest a significant potential for bioconcentration of fenac. A low octanol-water partition coefficient for fenac may explain the low bioaccumulation of the herbicide in fish. Neely, Branson, and Blau (8) reported a linear relationship between the octanol-water partition coefficient for a chemical and its ability to bioaccumulate in fish.

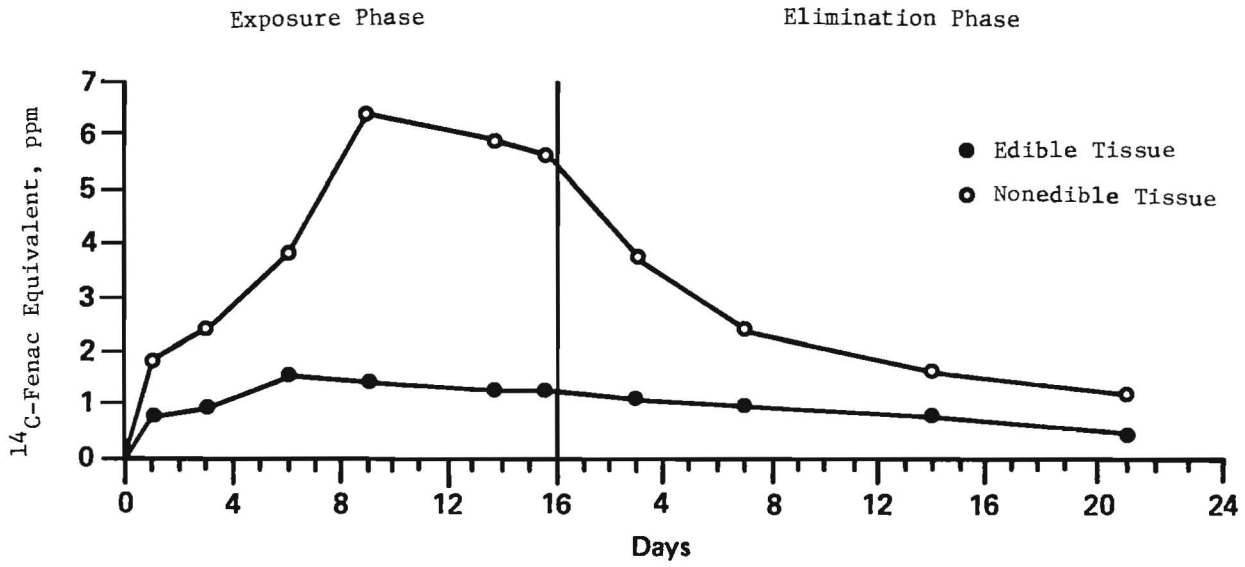


Figure 14. Uptake and elimination of ^{14}C-fenac in bluegill sunfish

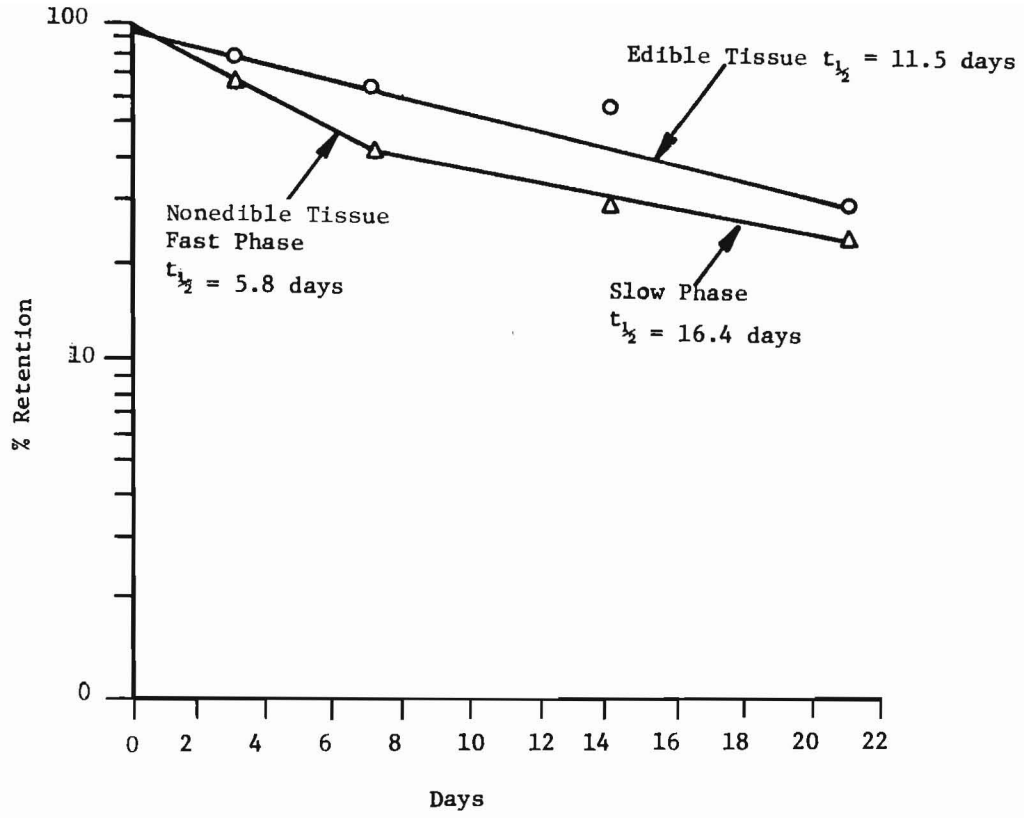


Figure 15. Elimination of ^{14}C-fenac from bluegill sunfish

Table 20

Bioaccumulation of ^{14}C-Fenac by Bluegills

Exposure (hours)	Tissue Fraction	ppm ^{14}C-Fenac Equivalent* In fish tissue	In water	BCF**
24	Edible	7.5 ± 0.67	1.30	5.77
	Nonedible	10.69 ± 1.51		8.22
72	Edible	8.89 ± 0.40	1.32	6.73
	Nonedible	13.73 ± 0.32		10.40
120	Edible	12.07 ± 0.14	1.42	8.50
	Nonedible	16.49 ± 0.46		11.61
168	Edible	11.20 ± 0.09	1.38	8.12
	Nonedible	9.99 ± 1.64		7.24
240	Edible	11.43 ± 0.65	1.40	8.16
	Nonedible	15.886 ± 0.02		11.35

* Values are the average of duplicate analysis, with two fish per analysis

** Bioconcentration factor - concentrations of ^{14}C in fish/concentrations of ^{14}C in water.

Metabolism of fenac by bluegills

95. In a separate experiment, bluegills were exposed to 2 ppm ^{14}C-fenac of high specific activity (3.1 μ Ci/mg) for 7 days in a static system, at which time both the water and fish were analyzed for fenac and potential metabolites. Analysis of methanol extracts of fish and ether extracts of water by silica-gel thin-layer chromatography in two solvent systems: (a) chloroform:acetic acid (9:1), and (b) chloroform showing radioactive spots with R_f values corresponding to authentic fenac (R_f of 0.47 and 0.10, respectively). The lack of metabolites was confirmed by gas-chromatography of samples after methylation, which suggested that all of the ^{14}C in the fish was in the form of fenac.

Bioaccumulation of Fenac by Catfish

96. During the 30-day aging period, the level of ^{14}C-fenac in water remained relatively constant. After adding the fish, the level of total radio-

activity in the water showed little variation between sampling dates over a 21-day period (Table 21). The ^{14}C-material in the water (through 21 days after adding the fish) was found to be fenac as determined by GLC analysis.

Table 21

Residues of Fenac in Water in Aquaria Containing Water, Sediment, and Catfish

Days (after introducing Fish)	Tank	Fenac Concn. in Water (ppm)
0	1	1.88
0	2	2.06
1	1	1.91
1	2	1.93
3	1	1.83
3	2	1.83
7	1	1.86
7	2	1.84
10	1	1.82
10	2	1.88
14	1	1.84
14	2	1.82
21	1	1.81
21	2	1.95
28	1	1.83
28	2	1.82

97. The levels of ^{14}C in catfish at various times after exposure to ^{14}C-fenac are shown in Table 22. The ^{14}C-residue data shown in Table 22 are based on the total level of ^{14}C in the fish obtained by combusting the fish exposed to ^{14}C-fenac and counting the resulting $^{14}CO_2$. The absolute levels of fenac in the fish were not determined because of low levels of ^{14}C-residues. The results clearly show that the uptake of fenac by the fish is quite low. The concentration of ^{14}C-residues (^{14}C-fenac equivalents) in fish was less

than that in the ambient water and appeared to have reached equilibrium within 24 hours after adding the fish. These results confirm the earlier findings with bluegill sunfish that fenac does not bioaccumulate in fish.

Table 22
Uptake of ^{14}C-Fenac by Catfish*

Days (after introducing fish)	Tank	Whole fish ^{14}C-fenac equivalent/ g wet wt.	Viscera ^{14}C-fenac equivalent/ g wet wt.	Head ^{14}C-fenac equivalent/ g wet wt.	Edible Flesh ^{14}C-fenac equivalent/ g wet wt.
11	1	0.439	0.86	0.67	0.40
12	2	0.884	0.40	0.29	0.33
31	1	0.338	0.78	0.35	0.36
32	2	0.632	0.72	0.34	0.32
71	1	0.492	0.50	0.28	0.24
72	2	0.308	0.64	0.10	0.14
101	1	0.710	0.81	0.46	0.31
102	2	0.344	0.64	0.1	0.14
141	1	0.588	0.82	0.33	0.28
142	2	0.256	0.43	0.08	0.20
211	1	1.02	1.2	0.11	0.25
212	2	0.437	0.42	0.09	0.175

* Values are the average of duplicate analysis, with two fish per analysis.

Bioaccumulation of Fenac by Daphnia

98. The results on the uptake of ^{14}C-fenac by Daphnia are given in Table 23. The ^{14}C-residue level reached equilibrium within 24 hours, with a BCF of 2.1 achieved (average of 24- to 72-hr values). These data show that fenac will not accumulate in fish by food chain magnification.

Table 23

Bioconcentration of ^{14}C-Fenac by Daphnia magna

Time (hr)	^{14}C-Fenac/g Daphnia (fresh weight)*
4	2.05
24	4.47
48	4.04
72	4.08

* Values are the average of two experiments, each within two replications. The exposure medium contained 2 ppm ^{14}C-fenac.

99. Results on uptake of fenac by fish and Daphnia show that the potential for bioaccumulation of the herbicide in aquatic organisms is quite low. A lower uptake of fenac by these organisms may be explained by the fact that the herbicide in the water is mostly present in an ionized form (pK $\sim$ 3.7) which is less likely to partition from water into the aquatic organisms.

CONCLUSION

100. On the basis of the results of these studies, it appears that the biological and nonbiological processes examined in this investigation are not likely to influence the environmental fate of fenac. The herbicide is expected to persist in an aquatic ecosystem because of its resistance to biological and nonbiological degradation. However, in spite of its tendency to persist, fenac may not present an environmental hazard because of its low potential for bioaccumulation and low toxicity to aquatic organisms.

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PART III: HEALTH HAZARD EVALUATION OF FENAC

MATERIALS AND METHODS

Chemicals

101. The ^{14}C-fenac (∿ 99 percent, 2,3,6-trichlorophenyl acetic acid, uniformly ring-labelled) and nonradioactive fenac used in this study were provided by Amchem Products, Inc. A solution of the herbicide with a specific activity of 0.2 μ Ci/mg was prepared by dissolving appropriate amounts of radioactive and nonradioactive fenac in dimethylsulfoxide (DMSO) and polyethylene glycol 300 (PEG) in a volumetric ratio of 1:7.

Laboratory Tests

Experimental animals

102. Sprague-Dawley male rats weighing 225-300 g were used in these studies.

Absorption and excretion of fenac

103. The animals were fasted for about 24 hours prior to administering fenac, but were allowed free access to water. A dosage of 49.9 mg ^{14}C-fenac [1.83×10^7 dpm in 1 ml of DMSO:PEG 300 (1:7)] was administered to each rat by oral intubation or intraperitoneally. After dosing, the animals were housed in individual metabolism cages which permit separate collection of urine and feces. The animals were allowed free access to food and water four hours after administering the herbicide.

104. Urine and feces samples were collected at appropriate intervals through 142 hours after administration of ^{14}C-fenac. At each collection period, duplicate 50-μl urine samples were radioassayed directly by liquid scintillation counting in scintillation cocktail and the remaining samples were frozen for later analysis. The fecal samples were homogenized with added water to give a uniform paste. The total radioactivity in the feces

was determined by combusting weighed aliquots of the fecal homogenate in a Packard Sample Oxidizer and counting the radioactivity of the resulting $^{14}CO_2$.

Tissue distribution

105. The animals were fasted for about 24 hours prior to administering ^{14}C-fenac but were allowed free access to water. A single oral dose of 57.6 mg of ^{14}C-fenac in DMSO:PEG 300 (1:7) solution containing 2.0 μ Ci/ 0.025 ml was administered to each rat. After dosing, the animals were allowed food and water ad libitum. The animals were killed in groups of three at 24, 48, 72, and 96 hours after administering ^{14}C-fenac at which time various tissues were removed. The tissues were weighed, air-dried, and assayed for total radioactivity by combusting them in a Packard Tri-Carb Sample Oxidizer counting the radioactivity in the liberated CO_2.

Pharmacokinetics of fenac in the plasma

106. The rats were fasted for a period of 12 to 24 hours prior to administering ^{14}C-fenac but were allowed free access to water. A single oral dose of 178 mg/kg (0.07 μ Ci/0.025 ml) was administered to each rat. After dosing, the animals were placed in stainless steel metabolic cages and allowed food and water ad libitum. The animals were killed by cervical dislocation in groups of three at 1/2, 1, 2, 4, 6, 8, 12, 16, and 24 hours after oral dosing of ^{14}C-fenac and the blood was withdrawn by heparinized syringes. The plasma and erythrocytes were separated by centrifugation at 3000 x g for 10 minutes. An aliquot of plasma was counted in a liquid scintillation counter to determine the total radioactivity. To determine the level of fenac, an aliquot of the plasma was diluted with 0.01 M phosphate buffer, pH 7.4 (1:1 v/v), adjusted to pH 2 with HCl, and extracted with diethyl ether. The ether extracts were dried under a stream of nitrogen, redissolved in methanol, and subjected to HPLC analysis. The appearance and disappearance of radio-

activity in the plasma was determined by a stripping technique (5) utilizing a computer program to decompose the pharmacokinetic data into individual first-order components.

Biliary excretion

107. The rats were surgically cannulated with polyethylene tubing (PE-10 inserted between the common bile duct and the duodenum (3)). The tubing was inserted under the skin through an incision at the nape of the neck. The incisions were sutured; the rats were placed in a restraining cage and allowed to recover for 24 hours. The rats were then administered orally 60 mg/kg of ^{14}C-fenac [2.34 x 10^7 dpm/mg in DMSO:PEG (1:7 v/v)]. The bile was collected periodically and aliquots were counted for radioactivity.

In vivo metabolism of fenac

108. The pooled urine or bile samples were diluted with distilled water, adjusted to ∿ pH 2 with HCl, and extracted with diethyl ether. The ether extract was dried under a stream of nitrogen and the residue was redissolved in methanol. The methanol extract was analyzed by HPLC using a Waters Associates liquid chromatograph equipped with a UV detector and a Flo-one radioactivity monitor. Analysis by HPLC was performed on a Waters μ Bondapak C_{18} reversed phase which was eluted with acetonitrile:4 percent acetic acid in water (1:1 v/v).

In vitro metabolism of fenac

109. The in vitro metabolism of fenac was examined using both a tissue homogenate (9000 x g supernatant fraction) and a microsomal fraction. In an attempt to enhance the metabolism of fenac, the herbicide was incubated with tissue homogenate or microsomes prepared from livers or kidneys of rats previously administered PCB's, which are known to induce the activity of microsomal enzymes. Rats were administered intraperitoneally Aroclor 1254 (25 mg/kg, once daily) for three consecutive days. The animals were killed 24 hours after the last injection. The tissue homogenate and microsomes were prepared according to published procedures (2,4).

110. Tissue homogenate. Freshly removed liver or kidney tissue was rinsed in cold (0-4°C) 0.05 M phosphate buffer (pH 7.4) containing 1.15% KCl. The tissue was minced with scissors and homogenized with 3 volumes of cold buffer in a glass Potter-type homogenizer with a motor-driven Teflon pestle. The homogenate was centrifuged at 9000 x g for 20 minutes and the supernatant (referred to as the tissue homogenate) was removed, kept at 4°C, and used as an enzyme source for the in vitro metabolism study. The reaction was carried out in a final volume of 25 ml containing 62.5 μmole $NADP^+$, 7.5 units glucose-6-phosphate dehydrogenase, 62.5 μmole $MgCl_2$, 50 mg liver or kidney microsomal protein, and 2.5 μmole ^{14}C-fenac dissolved in 50 μl DMSO. The reaction mixture was incubated at 37°C for 3 hours and then extracted with diethyl ether after adjusting the pH to about 2. The ether extract was evaporated, and the residue was redissolved in methanol and subjected to HPLC analysis.

111. Microsomal fraction. Liver microsomes were prepared by centrifuging the 9000 x g supernatant (tissue homogenate) at 105,000 x g for 60 minutes. The pellet was resuspended in the 0.1 M phosphate buffer (pH 7.4) and centrifuged a second time at 105,000 x g for 60 minutes; the washed microsomes were then suspended in a volume of the buffer to produce the desired protein concentration. The incubation mixture for studying the metabolism of fenac consisted of an NADPH-generating system, phosphate buffer, microsomal protein (1 mg/ml), and ^{14}C-labelled fenac dissolved in DMSO. The reaction mixture was incubated for one hour at 37°C and then analyzed for fenac and possible metabolites by HPLC.

In vitro mutagenicity of fenac

112. The Salmonella typhimurium strains used for the detection of mutagenic sactivity were obtained from Dr. Bruce Ames, University of California at Berkeley. Tests for mutagenicity were carried out according to the procedures developed by Ames et al. (1). To 3 ml of molten top agar at 45°C were

added 0.1 ml of the bacterial strain culture (1.5×10^8 cells), 0.1 of fenac solution in DMSO, and 0.5 ml of rat liver microsomal fraction ("S9") which was prepared according to Ames et al. (1). The control tubes contained 0.1 ml of DMSO. The contents of the tubes containing top agar were mixed and poured on agar plates. The plates were incubated for 48 hours at 37°C and the number of colonies which represent reverse mutants were counted. N-Methyl-N-nitro-n'-nitrosoguanidine and 2-acetylaminofluorene, which are known mutagenic compounds, were used as positive controls in each assay.

RESULTS AND DISCUSSIONS

Elimination in Urine and Feces

113. The cumulative excretion of radioacticity in the urine and feces of rats administered ^{14}C-fenac orally or intraperitoneally is shown in Figure 16. The data show that the administered herbicide was easily absorbed and then excreted both via the urine and feces. Within 70 hours after receiving a single oral or intraperitoneal dose of ^{14}C-fenac, the rats had excreted more than 80 percent of the administered radioactivity via the urine and feces. The primary route of radioactivity was via the urine after oral or intraperitoneal administration, a feature characteristic of most noncumulative pesticides. The bulk of the excretion in the urine occurred between 10-46 hours after dosing. During the initial 46 hours after dosing, 63% of the fenac was excreted via the urine in the orally treated rats. Another 11 percent of the dose was excreted in the feces during this period. In the rats given ^{14}C-fenac intraperitoneally, 59.5 and 17.7 percent of the total radioactivity had been eliminated via urine and feces, respectively. These findings show that, even at a relatively high dose of about 200 mg/kg, a single oral or intraperitoneal

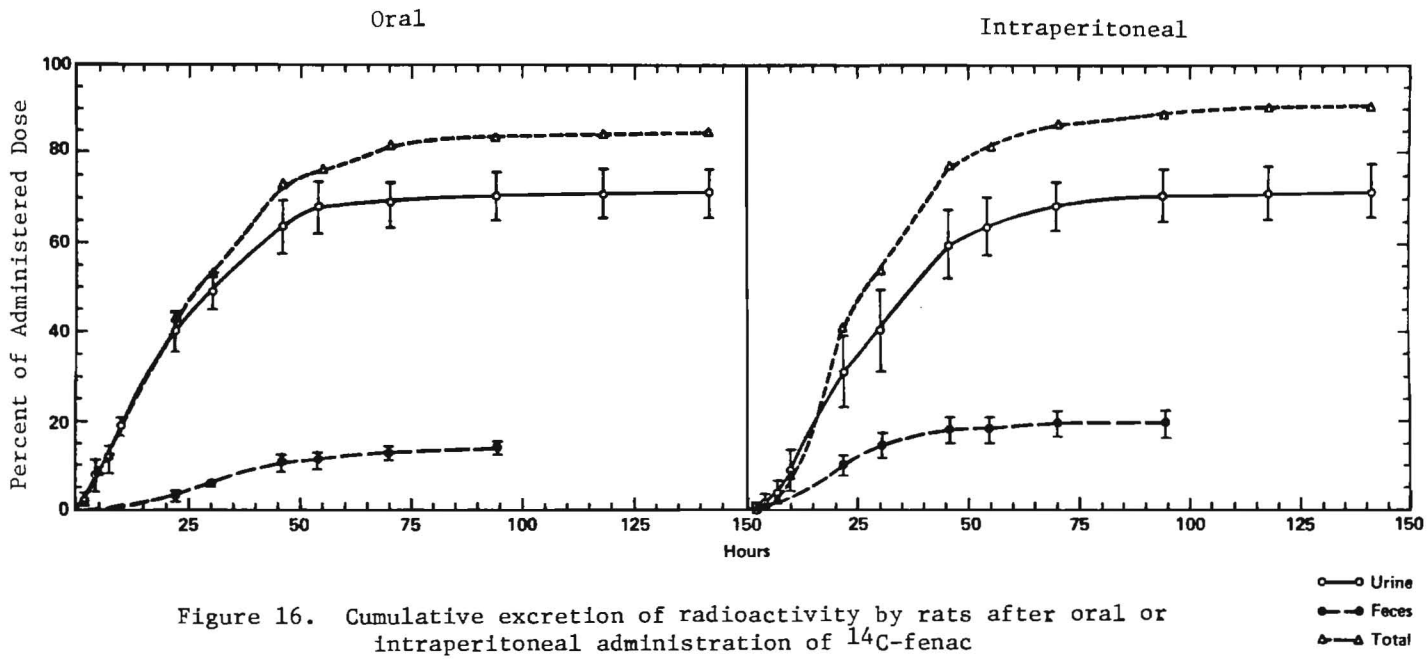

Figure 16. Cumulative excretion of radioactivity by rats after oral or intraperitoneal administration of ^{14}C-fenac

dose of ^{14}C-fenac is rapidly excreted, resulting in an elimination of approximately 85 percent of the administered ^{14}C-fenac in 70 hours.

Tissue Distribution of ^{14}C-Fenac and Metabolites

114. The distribution of radioactivity in various tissues following a single oral dose of ^{14}C-fenac is shown in Table 24. Appreciable radioactivity was detected in all tissues at 24 hours indicating a rapid absorption and distribution of ^{14}C-fenac and its metabolites. The principal sites of distribution of fenac-derived radioactivity were liver, lung, kidney, and gastrointestinal tract. The concentration of ^{14}C in the tissues continued to decline between 24 hours and 96 hours. After 24 hours of dosing, the radioactivity in the G.I. tract accounted for about 36 percent of the total ^{14}C-dose. Subsequently, the ^{14}C in the G.I. tract declined rapidly so that, after 72 hours, the G.I. tract contained less than one percent of the initial ^{14}C.

Biliary Excretion

115. To evaluate the importance of the biliary excretion route and its potential for enterohepatic circulation of fenac and its metabolites, the excretion and metabolism of fenac was studied in bile-duct cannulated rats (3). After 10 hours of dosing, about 86 percent of the administered radioactivity was excreted in the bile in the bile-duct cannulated rats (Table 25). Since only about 3.3 percent of the dose was excreted in feces in normal rats after 22 hours of dosing the animals, the results suggest most of the radioactivity excreted in the bile was reabsorbed by the intestinal mucosa and then excreted through the urinary tract.

Table 24

Distribution of Radioactivity in Rat Tissues Following Oral Administration of ^{14}C-Fenac

Tissue	24 Hours		48 Hours		72 Hours		96 Hours	
	Concentration of ^{14}C-Fenac Equivalent (µg/g tissue)	Percent of Administered Dose	Concentration of ^{14}C-Fenac Equivalent (µg/g tissue)	Percent of Administered Dose	Concentration of ^{14}C-Fenac Equivalent (µg/g tissue)	Percent of Administered Dose	Concentration of ^{14}C-Fenac Equivalent (µg/g tissue)	Percent of Administered Dose
Heart	18.04 ± 1.01	0.033 ± 0.005	3.13 ± 1.47	0.005 ± 0.002	0.777 ± 0.231	0.0013 ± 0.00058	0.473 ± 0.105	<0.001%
Lung	21.67 ± 3.34	0.055 ± 0.012	4.31 ± 1.456	0.009 ± 0.004	1.190 ± 0.258	0.003 ± 0.001	0.650 ± 0.195	0.0017 ± 0.0006
Pancreas	11.11 ± 4.13	0.068 ± 0.098	1.922 ± 1.591	0.0024 ± 0.0020	0.812 ± 0.345	<0.001%	0.507 ± 0.132	<0.001%
Spleen	8.56 ± 0.73	0.008 ± 0.001	1.627 ± 0.586	0.0016 ± 0.0007	0.587 ± 0.148	<0.001%	0.477 ± 0.091	<0.001%
Kidney	52.22 ± 5.28	0.205 ± 0.004	21.260 ± 8.421	0.069 ± 0.035	4.56 ± 0.89	0.019 ± 0.004	2.637 ± 0.492	0.009 ± 0.001
Testis	9.40 ± 1.00	0.057 ± 0.011	3.670 ± 3.642	0.011 ± 0.0046	0.467 ± 0.146	0.003 ± 0.001	0.460 ± 0.272	0.0026 ± 0.0021
Liver	57.52 ± 7.53	1.002 ± 0.197	9.117 ± 4.185	0.161 ± 0.050	3.590 ± 1.395	0.068 ± 0.018	2.827 ± 0.820	0.043 ± 0.006
Stomach + contents	494.57 ± 242.95	5.392 ± 2.551		0.737 ± 1.139	2.935 ± 2.062	0.027 ± 0.003	3.663 ± 2.653	0.017 ± 0.009
Sm. Int. + contents	1140.02 ± 384.54	15.262 ± 4.032	118.09 ± 60.22	1.605 ± 0.377	13.423 ± 7.981	0.230 ± 0.106	5.317 ± 3.653	0.074 ± 0.041
Caeca + contents	1329.10 ± 837.51	12.149 ± 5.876	79.563 ± 51.564	0.898 ± 0.518	15.880 ± 13.386	0.164 ± 0.080	4.347 ± 2.771	0.038 ± 0.020
L. Int. + contents	640.66 ± 385.38		23.583 ± 9.277	0.117 ± 0.077	11.220 ± 8.984	0.074 ± 0.072	3.003 ± 1.794	0.017 ± 0.011
Muscle	13.46 ± 7.05	3.387 ± 1.758	1.334 ± 0.574	0.221 ± 0.308	0.567 ± 0.214	0.0017 ± 0.0012	0.447 ± 0.120	0.0016 ± 0.0006
Bone	12.98 ± 3.36	0.495 ± 0.128	1.720 ± 0.342	0.024 ± 0.037	1.26 ± 0.717	<0.001%	0.762 ± 0.110	<0.001%
Eye	2.89 ± 1.42	0.003 ± 0.001	1.077 ± 0.712	<0.001%	0.902 ± 0.106	<0.001%	0.767 ± 0.172	<0.001%

Table 25

Biliary Excretion of Radioactivity in Male Rats Following Oral Administration of ^{14}C-Fenac

Time (hours)	Concentration of ^{14}C-Fenac Equivalents (mg/ml bile)	Percent of Administered Dose
0.5	2.91	2.57
1.5	6.19	13.89
2.5	12.56	32.88
4.0	8.10	55.90
6.0	5.03	85.94

Kinetics of ^{14}C-Fenac-Derived Radioactivity in Plasma

116. The concentrations of radioactivity in the plasma of rats at various times following an oral dose of ^{14}C-fenac (178 mg/kg) are shown in Figure 17. The concentrations of ^{14}C-fenac-derived radioactivity and the parent compound in the plasma reached a peak at 2-4 hours after dosing, indicating a rapid absorption of herbicide. The concentration of ^{14}C-fenac and its metabolites gradually declined thereafter in a biphasic manner. The radioactivity data for the plasma can be expressed as a summation of three exponential terms including a first-order availability process coupled with a two-compartment open model (5):

$$C = Ad^{-\alpha t} + Be^{-\beta t} - De^{-K_A t}$$

where K_A is the first-order availability rate constant, α and β are first-order elimination constants for the rapid and slower phases, respectively. Analysis of the plasma radioactivity according to this relationship gave half-life values for the different processes which are presented in Table 26. The half-lives for the rapid phases (α) of elimination were 0.85 and 0.82 hours for fenac and ^{14}C-fenac-derived radioactivity, respectively. The

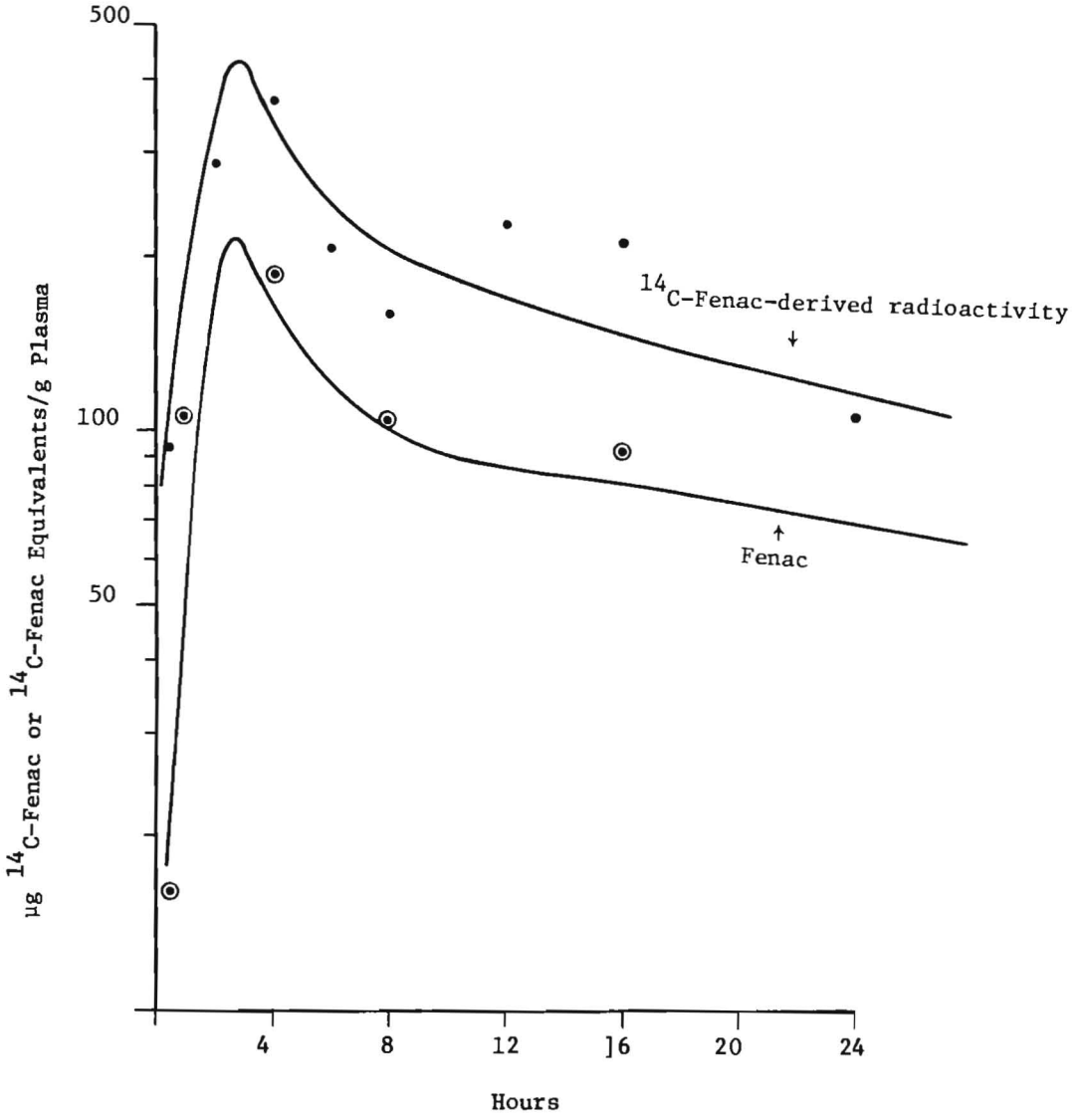

Figure 17. Concentration of fenac and ^{14}C-fenac-derived radioactivity in the plasma of rats receiving a single dose of ^{14}C-fenac

half-lives for the slower phases (β) of elimination were 30.1 and 23.1 hours for fenac and total ^{14}C-fenac-derived radioactivity, respectively.

Table 26

Pharmacokinetic Parameters for Radioactivity in Plasma of Rats Dosed Orally with ^{14}C-Fenac

	Half-life (Hours) α	β	K_A	Exponential Expression
Fenac	0.85	30.13	0.74	$C = 1932.13e^{-0.815t} + 118.84e^{-0.023t} - 2050.96e^{-0.934t}$
Total ^{14}C	0.82	23.12	0.75	$C = 4168.76e^{-0.845t} + 247.38e^{-0.030t} - 4416.13e^{-925t}$

Metabolism of Fenac

In vivo metabolism

117. Only the urine and bile were examined for metabolites as they represented the major routes of excretion. Essentially, all of the radioactivity in the urine and bile was present in the ether extract (pH 2). Analysis of urine from rats treated with ^{14}C-fenac showed six metabolites (Table 27), which accounted for more than 85 percent of the total radioactivity in the urine extract. Among the metabolites formed, only two (B and C in Table 28) were the major ones; each of the major metabolites contained about 35 percent of the ^{14}C. These findings suggest that fenac is extensively metabolized by rats.

118. HPLC analysis of the ether extracts of the bile showed the presence of ^{14}C-fenac and two metabolites (A and B in Table 28). The two metabolites accounted for about 85 percent of the radioactivity in the extract, suggesting that fenac was rapidly metabolized in the liver and the metabolites were excreted in the bile. The findings indicate that the fenac metabolites reaching the intestine via the biliary system may be reabsorbed by the intestinal mucosa and then excreted through the urinary

route. Due to time constraints, the metabolites could not be isolated and collected in amounts sufficient for characterizing them.

In vitro metabolism

119. No metabolites of fenac were detected upon incubation of the herbicide with liver microsomes. However, three ^{14}C-metabolites [one major (A) and two minor (C and D)] were formed when ^{14}C-fenac was incubated with a rat liver tissue homogenate (9000 x g) supernatant fraction (Table 28). The liver homogenate appeared to be more active in metabolizing fenac than the kidney homogenate. Incubation of ^{14}C-fenac with kidney homogenate resulted in the formation of only one metabolite. These results show that the soluble enzymes, and not the microsomal enzymes, are responsible for biotransformation of fenac in the liver.

Mutagenicity of Fenac

120. The authors investigated whether technical-grade fenac (70 percent as 2,3,6-trichlorophenyl acetic acid) and analytical-grade fenac (about 90 percent as 2,3,6-trichlorophenyl acetic acid) can induce mutations in histidine-requiring strains of Salmonella typhimurium (TA1535, TA98, TA100, and TA1537) with and without a rat liver microsomal activation system (Ames test). The results presented in Table 28 show that both the technical-grade and analytical-grade fenac were inactive in inducing mutations in the Salmonella strains.

Table 27

In Vivo and In Vitro Metabolism of ^{14}C-Fenac in Rats

	Relative Distribution of ^{14}C-Fenac and Metabolites						
Sample	A	B	C	D	E	F (fenac)	G
Urine (0-24 hrs.)	7.3* (2.6)**	34.8 (4.0)	36.5 (5.1)	5.0 (6.1)	1.7 (7.5)	6.1 (9.1)	8.1 (14.0)
(24-48 hrs.)	7.8 (2.6)	29.7 (4.0)	17.4 (5.1)	1.2 (6.0)	1.9 (7.5)	7.1 (9.1)	34.9 (14.0)
Bile (0-10 hrs.)	20.0 (2.7)	64.7 (4.0)	-	-	-	15.3 (8.7)	-
Kidney (S-9)	4.7 (3.0)	-	-	-	-	84.0 (9.5)	10.0 (14.0)
Liver (S-9)	37.9 (2.9)	-	0.3 (5.0)	0.5 (5.8)	-	61.3 (8.8)	-

* The values represent percentages of the total radioactivity in each sample.

** The numbers in parentheses represent the HPLC retention volumes (ml) for the metabolites.

Table 28

Mutagenic Activity in Fenac in Salmonella typhimurium

Chemical	Amount µg/plate	Mutants per plate* TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA1535 -S9	TA1535 +S9	TA1537 -S9	TA1537 +S9
Experiment #1	0	12	26	121	69	1	3	8	43
Analytical-grade Fenac (90%)	125	5	23	132	73	0	2	8	28
	250	5	26	116	79	0	3	8	42
	500	6	26	130	43	0	5	7	47
	750	8	24	116	66	0	1	5	16
MNNG**	2					1100			
2-AF+	10	78	1155	166	686				
Experiment #2									
Technical-grade Fenac (70%)	0	3	13			6	9		
	125	10	9			6	8		
	250	4	7			6	13		
	500	5	6			3	11		
	750	6	7			2	8		
Analytical-grade Fenac (90%)	0	5	8			10	10		
	125	10	9			6	13		
	250	4	9			9	11		
	500	6	12			6	10		
	750	6	11			6	11		
MNNG	2					>1000			
2-AF	10	83	>1000						

* Each number is the average of two replicate plates.

** N-Methyl-N-nitro-n'-nitrosoguanidine.

\+ 2-acetyl-aminofluorene

CONCLUSION

121. Fenac is readily absorbed, metabolized, and excreted via the urine and feces. This rapid metabolism of fenac may account for the relative resistance of the rat to the acute toxicity of the herbicide.

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APPENDIX A: RAW DATA FROM WHICH THE VALUES PRESENTED IN THE TABLES AND FIGURES IN THE TEXT WERE DERIVED

Table 1

Adsorption of Fenac to Sediment

Time hours	Flask number[1]	fenac Conc. (ppm)	µg fenac adsorbed	µg fenac adsorbed / g sediment
0	ALL	2.00	0.0	0.0
2	1	1.96	1.8	3.6
	2	1.98	1.1	2.2
	4	1.94	3.1	6.2
	5	1.96	2.2	4.4
	7	1.95	2.5	5.0
	8	1.96	2.0	4.0
	10	1.98	0.9	1.8
	11	1.97	1.3	2.6
	BLANK	2.00	Ø.Ø	Ø
5	1	1.95	2.6	5.2
	2	1.94	3.1	6.2
	4	1.92	4.2	8.4
	5	1.91	4.6	9.2
	7	1.91	4.6	9.2
	8	1.93	3.6	7.2
	10	1.96	1.6	2.4
	11	1.95	2.7	5.4
	BLANK	2.01	Ø	Ø
24	1	1.89	4.5	9.0
	2	1.92	4.7	9.4
	4	1.85	7.6	15.2
	5	1.85	7.5	15.0
	7	1.88	6.0	12.0
	8	1.89	5.4	10.8
	10	1.90	4.9	9.8
	11	1.94	2.9	5.8
	BLANK	1.96	2.1	4.2

[1] Flasks #1 and 2 contained organic muck; flasks #4 and 5, oxidized clay; flasks #7 and 8, reduced clay; and flasks #10 and 11, sandy sediment.

Corresponds to Figure 1 and Table 3 in the text.

Table 2

Effect of pH on the Adsorption of Fenac to Sediment, pH 4.5

Time hours	Flask number[1]	fenac conc. (ppm)	µg fenac adsorbed	µg fenac adsorbed/ g sediment
0	ALL	2.00	0.0	0.0
3	1	1.77	.23	11.5
	2	1.81	.19	9.5
	4	1.77	.23	11.5
	5	1.84	.16	8
	7	2.02	-	-
	8	1.84	.16	8
	10	1.96	.04	2
	11	1.93	.07	3.5
	BLANK	1.80	.20	10
6	1	1.79	.21	10.5
	2	1.81	.19	9.5
	4	1.83	.17	8.5
	5	1.84	.16	8
	7	1.80	.20	10
	8	1.79	.21	10.5
	10	1.90	.10	5
	11	1.93	.07	3.5
	BLANK	1.98	.02	1.0
9	1	1.76	.24	12
	2	1.80	.20	10
	4	1.87	.13	6.5
	5	1.84	.16	8.0
	7	1.79	.21	10.5
	8	1.78	.22	11.0
	10	1.88	.12	6.0
	11	1.95	.05	2.5
	BLANK	1.97	.03	1.5
24	1	1.79	.21	10.5
	2	1.77	.23	11.5
	4	1.82	.18	9.0
	5	1.81	.19	9.5
	7	1.71	.29	14.5
	8	1.75	.25	12.5
	10	1.91	.09	4.5
	11	1.90	.10	5
	BLANK	1.97	.03	1.5

[1] Flasks #1 and 2 contained organic muck; flasks #4 and 5, oxidized clay; flasks #7 and 8, reduced clay; and flasks #10 and 11, sandy sediment.

Corresponds to Figure 3 and Table 4 in the text.

Table 3

Effect of pH on the Adsorption of Fenac to Sediment, pH 9

Time hours	Flask number[1]	fenac conc. (ppm)	µg fenac adsorbed	µg fenac adsorbed/ g sediment
0	ALL	2.00	0.0	0.0
2	1	1.94	3	6
	2	1.96	2	4
	4	1.92	4	8
	5	1.94	3	6
	7	1.91	4.5	9
	8	1.92	4	8
	10	1.97	1.5	3
	11	1.94	3	6
	BLANK	1.97	1.5	3
5	1	1.94	3	6
	2	1.92	4	8
	4	1.93	3.5	7
	5	1.93	3.5	7
	7	1.91	4.5	9
	8	1.95	2.5	5
	10	1.95	2.5	5
	11	1.99	0.5	1
	BLANK	1.98	1	2
8	1	1.90	5.0	10
	2	1.92	4	8
	4	1.93	3.5	7
	5	1.93	3.5	7
	7	1.93	3.5	7
	8	1.93	3.5	7
	10	1.93	3.5	7
	11	1.97	1.5	3
	BLANK	1.99	.5	1
24	1	1.91	4.5	9
	2	1.91	4.5	9
	4	1.90	5.0	10
	5	1.92	4.0	8
	7	1.91	4.5	9
	8	1.93	3.5	7
	10	1.88	6	12
	11	1.94	3	6
	BLANK	1.97	1.5	3

[1] Flasks #1 and 2 contained organic muck; flasks #4 and 5, oxidized clay; flasks #7 and 8, reduced clay; and flasks #10 and 11 sandy sediment.

Corresponds to Figure 3 and Table 4 in the text.

Table 4

Adsorption Isotherm for Fenac in Organic Muck Sediment at pH 4.5

Time hours	Flask[1] number	fenac conc. µg/ml	µg fenac adsorbed	µg fenac adsorbed/ g sediment
18	1	0.086	0.7	1.4
	2	0.086	0.7	1.4
	3	9.26	37	74
	4	9.24	38	76
	5	9.19	40.5	81
	6	17.4	130	260
	7	17.5	125	250
24	1	0.0877	0.615	1.33
	2	0.0867	0.665	1.33
	3	9.19	40.5	81
	4	9.12	44	88
	5	9.02	49	98
	6	17.4	130	260
	7	17.4	130	260
42	1	0.0872	0.64	1.28
	2	0.0877	0.615	1.23
	3	9.11	44.5	89
	4	8.97	51.5	103
	5	9.11	44.5	89
	6	17.5	125	250
	7	17.2	140	280
48	1	0.0862	0.69	1.38
	2	0.0862	0.69	1.38
	3	9.11	44.5	89
	4	9.02	49	98
	5	9.02	49	98
	6	17.4	130	260
	7	17.4	130	260

[1] number 1,2 = 0.1 ppm Fenac
3,4,5 = 10.0 ppm Fenac
6,7 = 20.0 ppm Fenac

Corresponds to Figure 2 in the text.

Table 5

Hydrolysis of Fenac

Time	pH	Temperature	Fenac, ppm	Fenac, Average ppm ± S.D.
Ø	5	1/	2.49	2.52 ± .04
	5		2.54	
	7		2.59	2.46 ± .17
	7		2.34	
	9		2.44	2.54 ± .14
	9		2.64	
3 wks	5	25°	2.10	2.16 ± .08
	5	"	2.22	
	7	"	2.1	2.07 ± .04
	7	"	2.04	
	9	"	2.04	2.19 ± .21
	9	"	2.34	
	5	10°	2.46	2.5 ± .08
	5	"	2.34	
	7	"	2.10	2.13 ± .04
	7	"	2.16	
	9	"	2.22	2.22 ± 0
	9	"	2.32	
6 wks	5	25°	2.42	2.31 ± .13
	5	"	2.23	
	7	"	2.23	2.23 ±
	7	"	2.23	
	9	"	2.23	2.45 ± .34
	9	"	2.71	
	5	10°	2.42	2.42 + Ø
	5	"	2.42	
	7	"	2.23	2.35 ± .13
	7	"	2.42	
	9	"	2.62	2.62 ± Ø
	9	"	2.62	

1/ Aliquots of a common zero-time solution were used to determine hydrolysis at different temperatures.

Corresponds to Table 5 in the text.

Table 6

The pH Dependence of Fenac Photodegradation at 254 nm

Time	Peak height, arbitrary units 50 µℓ injected	60 µℓ injected	Average peak height/ µg injected[2]	Fenac, µg/ml
pH 2				
Ø[1]	80	86	1.52	100
30	74.5	81	1.42	93.4
60	69.5	77	1.34	88.2
85	68.5	74.5	1.30	85.5
130	62	68	1.18	77.6
pH 7				
Ø[1]	80	88	1.54	100
30	69	78	1.34	87.0
60	62	67	1.18	76.6
85	54	61.5	1.05	68.2
130	47	53.5	0.92	59.7
pH 9				
Ø[1]	78	85.5	1.48	100
36	66.5	79	1.32	89.2
66	61	76	1.20	81.1
85	56	63	1.08	73.0
130	45	54	0.90	60.8

[1] time zero = 100 ppm fenac

[2] one peak height unit/µl injected = 67 $\pm$ 1.3 µg/ml fenac

Corresponds to Figure 4 in the text.

Table 7

Degradation of Fenac in Water and Sediment Under Anaerobic Conditions

Time, wks	Aliquot, ppm	Average ppm
Ø	1.52 1.42	1.47
4	1.54 1.39	1.47
6	1.49 1.42	1.46
10	1.14 1.27	1.21
13	1.29 1.39	1.34
18	1.66 1.45	1.55
22	1.39 1.50	1.45

Corresponds to Table 8 in the text.

Table 8

Fenac Residues in Water in Aquaria Treated with 2 ppm of the Herbicide

Time, Wks	Sandy		Oxidized Clay		Reduced Clay		Organic Muck	
	ppm		ppm		ppm		ppm	
	Aliquot	Mean	Aliquot	Mean	Aliquot	Mean	Aliquots	Mean
1	1.38	1.37	1.21	1.21	0.96	.94	1.23	1.19
	1.37		1.215		0.92		1.17	
2	0.90	0.95	1.19	1.18	1.01	1.00	1.38	1.36
	1.00		1.18		1.00		1.34	
3	1.79	1.79	0.92	0.93	1.36	1.38	1.68	1.70
	1.79		0.95		1.39		1.71	
4	1.55	1.57	1.16	1.18	0.96	0.96	0.98	0.99
	1.58		1.20		0.97		1.00	
5	1.45	1.44	-	-	1.18	1.16	0.79	0.80
	1.44		-		1.14		0.80	
7	1.85	1.78	1.21	1.22	1.26	1.26	1.06	1.06
	1.71		1.23		1.26		1.05	
9	0.699	0.78	1.24	1.22	1.14	1.13	0.94	0.91
	0.775	0.79	1.21		1.12		0.87	
11	1.29	1.32	1.04	1.04	0.93	0.93	0.956	0.94
	1.35		1.05		0.93		.93	
14	1.42	1.44	0.93	0.91	0.71	.75	1.05	1.03
	1.46		0.90		0.78		1.01	
17	1.39	1.38	0.74	0.72	0.91	0.91	0.80	0.81
	1.38		0.70		0.92		0.81	
20	1.23	1.22	0.91	0.91	0.99	0.97	0.72	0.75
	1.20		0.90		0.96		0.78	
23	1.17	1.16	0.81	0.81	0.76	.75	0.87	0.88
	1.15		0.81		0.74		0.89	
25	1.01	1.02	0.83	0.82	0.69	.69	0.68	0.67
	1.03		0.81		0.68		0.66	
27	0.92	.97	0.76	0.75	0.66	.62	0.52	0.50
	1.01		0.74		0.59		0.47	
30	0.85	.88	0.76	0.72	0.56	.53	0.51	0.53
	0.90		0.69		0.50		0.55	
35	0.64	0.68	0.45	0.50	0.20	0.25	0.410	0.45
	0.71		0.56		0.30		0.48	

Corresponds to Figure 6 in the text.

Zero-time concentration was 2 ppm

Table 9

Fenac Residues in Water in 2.8-ℓ Flasks Containing Water and Sediment

Time	Reduced Clay		Organic Muck	
	ppm, Aliquot	Average ppm	ppm, Aliquot	Average ppm
0		2.0[1]		2.0[1]
0.5 hrs.	1.78 1.81	1.80	None Sampled "	
1	1.70 1.72	1.71	1.89 1.83	1.86
2	1.94 1.85	1.89	1.98 1.95	1.96
3	1.99 1.98	1.98	1.80 1.89	1.86
6	1.61 1.50	1.55	1.81 1.75	1.78
1	1.33 1.24	1.28	1.35 1.43	1.39
3	1.27 1.33	1.30	1.15 1.08	1.12
5	1.20 1.22	1.21	1.12 1.08	1.10
6	1.09 1.04	1.06	0.910 0.901	0.906
10	0.915 0.928	0.921	0.804 0.815	0.809
12	0.903 0.895	0.899	0.824 0.839	0.831

[1] 2.0 ppm added at zero-time from a known stock solution.

Corresponds to Figure 7 in the text.

Table 10

Toxicity of Fenac to Fathead Minnow

Concentration µg fenac/ml	Replicate Number	Mortalities at 24 hrs	48 hrs	72 hrs	96 hrs	Total Mortalities at 96 hrs[1]
Control	1	0	0	0	0	0
	2	0	0	0	0	0
40	1	0	0	0	0	0
	2	0	0	0	1	1
30	1	1	0	0	1	2
	2	0	0	0	0	0
20	1	0	0	0	1	1
	2	0	0	0	0	0
10	1	0	0	0	1	1
	2	0	0	0	0	0
5	1	0	0	0	0	0
	2	0	0	0	0	0

[1] Five fish were used per replicate.

Test was conducted at 7.8 ± 0.2 pH; Dissolved Oxygen from 7.2 to 8.3.

Corresponds to Table 14 in the text.

Table 11

Acute 96-Hour Toxicity of Fenac to Rainbow Trout

Fenac Concentration, ppm	Survival
0.4	5/5
4.0	5/5
40.0 (1)	5/5
40.0 (2)	5/5
Control (1)	5/5
Control (2)	5/5

Conditions

1 fish per liter; temperature = 12° ± 0.5°C; dissolved oxygen = 9.5-10.0 ppm.

Corresponds to Table 15 in the text.

Table 12

Effect of Fenac on Daphnia magna Reproduction

Daphnia Number	Fenac Concentrations									
	Control		20 ppm		5 ppm		2ppm		0.5 ppm	
	Broods[1]	Young[2]	Broods	Young	Broods	Young	Broods	Young	Broods	Young
1	7	89	2	7	5	38	7	79	3	30
2	*		*		6	67	9	109	3	29
3	3	21	*		*		*		3	31
4	3	33	*		*		7	71	1	3
5	3	19	*		*		*		7	75
6	1	5	5	46	7	67	*		*	
7	5	42	*		*		*		*	
8	8	89	*		4	34	6	74	5	76
9	5	59	*		*		*		4	51
10	4	37	7	95	1	2	*		5	65
Total	40	394	14	148	23	208	29	333	31	360
	Mean Brood size ± S.D.									
	9.85 ± 4.48		0.157 ± 5.52		9.04 ± 5.23		11.48 ± 4.19		11.61 ± 3.73	

[1] number of broods born during test period

[2] number of young born during test period

* Adult died before 21 days

Corresponds to Table 17 in the text.

Table 13

Effect of 2.0 ppm Fenac on *Daphnia magna* Reproduction

Replicate[1] Number	Control		2 ppm Fenac	
	offspring	broods	offspring	broods
1	61	7	30	4
2	24	3	14	2
3	44	5	54	6
4	18	2	26	3
5	45	4	49	6
6	45	5	49	6
7	38	3	21	2
8	51	5	48	5
9	31	4	9	2
10	48	5	57	7
11	63	7	50	6
12	43	6	29	3
13	53	6	16	2
14	57	7	51	5
15	17	2	44	6
Total	638	71	547	65
	Mean number of offspring/brood			
	8.98		8.41	

Corresponds to Table 18 in the text.

[1] Each replicate represents one *Daphnia*

Table 14

The 96-Hour Toxicity of Fenac to Daphnia magna

Concentration µg Fenac/ml	Survivors[1] Replicate number 1	2	3	Mean number of survivors	% Mortality
75	2	1	0	1	95
60	0	1	0	.3	98.5
50	2	0	1	1	95
40	0	0	1	.3	98.5
35	4	7	6	5.7	71.5
30	9	8	11	9.3	53.5
25	11	13	13	12.3	38.5
20	14	18	19	17	15
10	16	17	17	16.7	16.5
Ø[2]	19	20	20	19.7	1.5

[1] 20 animals per replicate at Ø time

[2] control, no fenac added

Corresponds to Figure 12 in the text.

Table 15

Acute 96-Hour Toxicity of Fenac to Daphnia magna

Concentration ppm	Number of survivors Replicate Number 1	2	3	% Mortality
60	1	0	1	96.7
50	1	1	2	93.3
40	2	3	3	86.7
35	6	8	5	68.3
30	11	9	12	46.7
25	14	12	12	36.7
20	15	18	17	16.7
10	18	17	19	10.0
Control	20			0.0

Corresponds to Figure 12 and Table 16 in the text.

Table 16

Uptake and Elimination of ^{14}C-Fenac by Bluegill Sunfish

Exposure Days	Tissue	µg ^{14}C-fenac Equivalents	Tissue Wet weight (g)[1]	µg ^{14}C-fenac Equivalents/g tissue
		Uptake Phase		
1	Edible Flesh	5.257	6.105	0.86
	Head & Viscera	10.0285	5.748	1.744
3	Edible Flesh	5.333	5.604	0.952
	Head & Viscera	14.929	6.384	2.338
6	Edible Flesh	11.106	6.607	1.681
	Head & Viscera	21.28	5.661	3.7588
9	Edible Flesh	7.824	5.441	1.438
	Head & Viscera	40.88	6.291	6.498
14	Edible Flesh	4.254	3.368	1.263
	Head & Viscera	20.999	3.54	5.928
15	Edible Flesh	5.1435	3.740	1.373
	Head & Viscera	21.326	3.788	
		Depuration Phase		
3	Edible Flesh	6.439	5.8016	1.11
	Head & Viscera	11.63	3.1314	3.713
7	Edible Flesh	3.179	3.4182	0.93
	Head & Viscera	12.99	5.494	2.366
14	Edible Flesh	2.773	3.476	0.798
	Head & Viscera	4.907	3.044	1.612
21	Edible Flesh	1.227	3.2541	0.377
	Head & Viscera	3.774	2.8810	1.290

[1] 2 fish were dissected and combined prior to analysis for fenac, entry represents the sum of the weights of two fish

Corresponds to Figures 14 and 15 in the text.

Table 17

Bioaccumulation of ^{14}C-Fenac by Bluegills

Time (Hours)	Tissue Fraction	Fish Number	ppm ^{14}C-Fenac Equivalents in Fish Tissue		Bioconcentration Factor	
			Per Fish	Mean	Per Fish	Mean
24	Edible Flesh	1	6.935	7.50	5.339	5.77
		2	8.065		6.209	
	Head & Viscera	1	12.202	10.69	9.393	8.22
		2	9.178		7.065	
72	Edible Flesh	1	9.290	8.89	7.011	6.73
		2	8.493		6.410	
	Head & Viscera	1	13.412	13.73	10.122	10.40
		2	14.049		10.603	
120	Edible Flesh	1	11.928	12.07	8.394	8.50
		2	12.21		8.592	
	Head & Viscera	1	17.25	16.49	12.139	11.61
		2	15.728		11.068	
168	Edible Flesh	1	11.29	11.20	8.181	8.12
		2	11.12		8.058	
	Head & Viscera	1	8.35	9.99	6.050	7.24
		2	11.632		8.428	
240	Edible Flesh	1	10.78	11.43	7.711	8.16
		2	12.08		8.644	
	Head & Viscera	1	15.906	15.89	11.378	11.35
		2	15.866		11.349	

Corresponds to Table 20 in the text.

Table 18

Bioconcentration of ^{14}C-Fenac by Daphnia magna, replicate[1]

Time (hr)	g Daphnia	µg ^{14}C-Fenac Equivalents/ g Daphnia	Mean
24	0.1366	4.309	4.0198
	0.7600	3.730	
48	0.0482	3.897	3.998
	0.0376	4.098	
72	0.0768	3.761	4.097
	0.0098	4.432	

Corresponds to Table 23 in text.

Bioconcentration of ^{14}C-Fenac by Daphnia magna, replicate [2]

Time (hr)	g Daphnia	µg ^{14}C-Fenac Equivalents/ g Daphnia	Mean
4	0.1070	2.042	2.049
	0.0153	2.056	
24	0.8857	4.700	4.365
	0.4086	4.030	
48	1.388	4.620	4.08
	0.4683	3.540	
72	1.045	4.338	4.072
	0.4887	3.806	

Corresponds to Table 23 in text.

Table 19

Residues of Fenac in Water in Aquaria Containing Water, Sediment, and Fish

Time	Tank 1 µg Fenac/ml	Tank 1 Mean ± S.D.	Tank 2 µg Fenac/ml	Tank 2 Mean ± S.D.
Ø	1.90	1.88 ± .029	1.98	2.06 ± .072
	1.90		2.11	
	1.85		2.10	
1	1.88	1.91 ± .026	1.93	1.93 ± .006
	1.93		1.92	
	1.92		1.93	
3	1.81	1.83 ± 0.15	1.82	1.83 ± .012
	1.84		1.82	
	1.83		1.84	
7	1.83	1.86 ± .038	1.83	1.84 ± .040
	1.84		1.88	
	1.90		1.80	
10	1.82	1.82 ± .06	1.90	1.88 ± .038
	1.88		1.91	
	1.76		1.84	
14	1.86	1.84 ± .025	1.83	1.82 ± .006
	1.84		1.82	
	1.81		1.82	
21	1.79	1.81 ± .025	1.92	1.95 ± .035
	1.81		1.95	
	1.84		1.99	
28	1.82	1.83 ± .036	1.84	1.82 ± .021
	1.87		1.86	
	1.80		1.83	

Corresponds to Table 21 in text.

Table 20

Uptake of ^{14}C-Fenac by Catfish

Time Days	Fish Portion[1]	Tank 1 µg ^{14}C-fenac Equivalents/g Tissue			Tank 2 µg ^{14}C-fenac Equivalents/g Tissue		
		Fish 1	Fish 2	Mean	Fish 1	Fish 2	Mean
1	V	1.51	0.377	0.944	1.16	1.19	1.22
	H	0.897	0.582	0.740	0.779	0.698	0.738
	EF	0.728	1.44	1.084	0.917	0.646	0.782
3	V	0.873	1.76	1.316	0.902	1.70	1.30
	H	0.218	0.981	0.600	0.658	0.563	0.610
	EF	0.300	0.345	0.324	0.430	0.572	0.501
7	V	0.426	1.27	0.848	0.971	1.34	1.16
	H	xxx	0.469	0.469	0.060	0.304	0.182
	EF	0.222	0.365	0.294	0.145	0.284	0.429
10	V	1.71	1.03	1.37	1.03	1.29	1.16
	H	1.20	0.369	0.784	0.063	0.299	0.181
	EF	0.787	0.252	0.520	0.078	0.425	0.252
14	V	1.44	1.48	1.46	0.799	0.747	0.647
	H	1.01	0.097	0.554	0.075	0.230	0.163
	EF	0.594	0.357	0.476	0.414	0.276	0.345
21	V	4.69	xxx	4.69	1.08	xx	1.08
	H	0.159	xxx	0.159	0.098	xx	0.098
	EF	0.431	xxx	0.431	0.138	xx	0.138

[1] V = Viscera
H = Head and Gills
EF = Edible flesh

Corresponds to Table 22 in the text

Table 21

Cumulative Excretion of Radioactivity by Rats After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Feces

Time	Animal number	Average dpm/g	Total dpm	Mean ± S.D.
24 hrs	3	No Feces		
	4	433,795	2.05×10^5	
	5	No Feces		$1.83 \times 10^6 \pm 407{,}000$
	6	467,602	1.36×10^6	
	7	899,683	2.08×10^6	
	8	148,467	5.81×10^5	
	9	204,461	5.99×10^5	$6.03 \times 10^5 \pm 2.78 \times 10^4$
	10	143,186	5.88×10^5	
	11	122,227	6.43×10^5	
	12	179,125	1.07×10^6	
32 hrs	3	494,622	1,253,867	$8.21 \times 10^5 \pm 4.72 \times 10^5$
	4	145,753	599,616	
	5	149,731	136,105	
	6	317,411	864,405	
	7	338,506	1,251,761	
	8	72,667	287,078	$3.32 \times 10^5 \pm 4.47 \times 10^4$
	9	72,050	339,586	
	10	91,903	283,687	
	11	77,306	366,347	
	12	119,845	381,106	

(Continued)

Table 21

Cumulative Excretion of Radioactivity by Rate After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Feces (continued)

Time	Animal number	Average dpm/gr	Total dpm	Mean ± S.D.
46 hrs	3	72,657	650,065	$5.91 \times 10^5 \pm 1.63 \times 10^5$
	4	44,781	360,307	
	5	51,187	520,281	
	6	111,626	802,816	
	7	101,814	621,878	
	8	67,624	700,263	$1.06 \times 10^6 \pm 6.03 \times 10^5$
	9	117,858	1,185,416	
	10	134,999	974,558	
	11	35,059	439,579	
	12	211,139	2,017,662	
54.5 hrs	3	381,345	287,153	$9.77 \times 10^4 \pm 109,000$
	4	15,221	15,221	
	5	179,371	34,260	
	6	39,262	66,549	
	7	52,357	85,499	
	8	56,962	110,278	$8.58 \times 10^4 \pm 2.83 \times 10^4$
	9	38,476	100,460	
	10	56,803	40,841	
	11	27,323	75,384	
	12	41,607	101,937	

(Continued)

Table 21

Cumulative Excretion of Radioactivity by Rats After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Feces (continued)

Time	Animal number	Average[1] dpm/g	Total dpm	Mean ± S.D.
70 hrs	3	50,678	2.95×10^5	$2.76 \times 10^5 + 1.72 \times 10^5$
	4	5,384	4.60×10^4	
	5	65,805	5.07×10^5	
	6	45,314	3.44×10^5	
	7	30,812	1.89×10^5	
	8	71,818	5.16×10^5	$2.72 \times 10^5 \pm 1.52 \times 10^5$
	9	23,435	1.42×10^5	
	10	33,129	3.15×10^5	
	11	16,915	1.59×10^5	
	12	26,285	2.29×10^5	
96 hrs	3	2,609	1.94×10^4	$4.96 \times 10^4 \pm 3.17 \times 10^4$
	4	2,666	3.41×10^4	
	5	31,661	3.81×10^5	
	6	4,132	5.23×10^4	
	7	10,254	9.28×10^4	
	8	20,421	2.33×10^5	$1.79 \times 10^5 \pm 1.17 \times 10^5$
	9	27,180	3.55×10^5	
	10	5,129	5.75×10^4	
	11	8,375	1.21×10^5	
	12	11,240	1.28×10^5	

(Continued)

Table 21

Cumulative Excretion of Radioactivity by Rats After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Feces (concluded)

Time	Animal number	Average dpm/g	Total dpm	Mean ± S.D.
118 hrs	3	2,860	1.53×10^4	$1.17 \times 10^4 \pm 4.52 \times 10^3$
	4	1,511	1.09×10^4	
	5	17,707	1.25×10^5	
	6	2,094	1.49×10^4	
	7	2,474	5.59×10^3	
	8	4,310	3.15×10^4	$2.35 \times 10^4 \pm 1.31 \times 10^4$
	9	1,300	9.39×10^3	
	10	2,635	2.05×10^4	
	11	1,620	1.44×10^4	
	12	4,758	4.18×10^4	

Corresponds to Figure 16 in the text.

Table 22

Cumulative Excretion of Radioactivity by Rats After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Urine

Animal # [1/]	Time	Total Rat	Mean ± S.D.
3	2 hrs.	90,900	58,170 ± 32,673
4		238,436*	
5		32,682	
6		81,566	
7		27,566	
8		414,948	481,810 ± 90,858
9		392,938	
10		-	
11		568,666	
12		551,088	
3	4.5 hrs.	282,124	283,945 ± 241,995
4		625,700	
5		137,517	
6		90,440	
7		<2,000*	
8		11,587,885	1,019,997 ± 436,266
9		1,197,610	
10		559,584	
11		1,155,529	
12		599,378	
3	7 hrs.	287,001	237,983 ± 88,596
4		667,702	
5		120,773	
6		322,488	
7		221,670	
8		737,332	710,617 ± 45,306
9		646,128	
10		712,593	
11		746,415	
12		926,431*	
3	10 hrs.	753,475	967,958 ± 377,623
4		1,546,562	
5		558,108	
6		1,092,285	
7		889,360	
8		1,020,026	1,283,001 ± 265,644
9		1,570,912	
10		1,569,095	
11		1,127,796	
12		1,127,178	

(Continued)

Table 22

Cumulative Excretion of Radioactivity by Rats After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Urine (continued)

Animal	Time	Total Rat	Mean ± S.D.
3	22 hrs.	4,889,340	4,087,699 ± 795,103
4		4,579,692	
5		3,951,859	
6		4,422,018	
7		3,595,590	
8		3,778,294	3,900,989 ± 466,082
9		4,445,121	
10		3,282,632	
11		4,278,773	
12		3,720,129	
3	30.5 hrs.	1,792,872	1,686,033 ± 307,930
4		1,673,325	
5		1,181,322	
6		2,011,086	
7		1,771,560	
8		1,088,672*	1,609,636 ± 398,939
9		1,629,537	
10		1,981,596	
11		2,004,432	
12		1,343,943	
3	46 hrs.	3,809,050	3,573,339 ± 1,164,652
4		2,000,600	
5		4,612,428	
6		4,663,718	
7		2,780,900	
8		2,684,878	2,519,467 ± 123,696
9		2,387,394	
10		2,519,271	
11		3,441,060*	
12		2,486,325	
3	54.5 hrs.	391,984	686,396 ± 471,547
4		173,866	
5		1,408,578	
6		635,877	
7		823,676	
8		51,331	410,266 ± 162,054
9		317,173	
10		232,875	
11		-	
12		577,684	

(Continued)

Table 22

Cumulative Excretion of Radioactivity by Rats After Oral or Intraperitoneal Administration of ^{14}C-Fenac; Urine (concluded)

Animal	Time	Total Rat	Mean ± S.D.
3	70 hrs.	434,967	848,720 ± 652,399
4		202,210	
5		1,825,740	
6		611,682	
7		1,169,000	
8		818,062	585,729 ± 250,687
9		298,357	
10		415,293	
11		524,753	
12		872,170	
3	94 hrs.	318,255	307,207 ± 75,826
4		214,272	
5		963,270*	
6		297,470	
7		398,832	
8		386,302	286,658 ± 165,413
9		143,734	
10		204,590	
11		169,750	
12		538,912	
3	118 hrs.	146,329	110,914 ± 39,032
4		108,100	
5		154,044	
6		80,264	
7		65,832	
8		95,440	57,258 ± 29,182
9		29,158	
10		48,760	
11		33,169	
12		79,763	
3	142 hrs.	70,291	66,494 ± 21,759
4		79,180	
5		93,280	
6		50,559	
7		39,162	
8		69,842	67,676 ± 44,006
9		19,278	
10		71,396	
11		41,595	
12		136,268	

1/ Animals numbered 1 & 2 were used as "blanks".
Animals numbered 3 - 7 were dosed intraperitonealy.
Animals numbered 8 - 12 were dosed orally.

* Not included in calculation for Mean or Stnd. Dev.

Corresponds to Figure 16 in the text.

Table 23

Pharmacokinetic Parameters for Radioactivity in Plasma of Rats Dosed Orally with ^{14}C-Fenac

Time (hrs)	Animal Number	^{14}C-Fenac Equivalents (µg/ml)		Fenac (µg/ml)	
		No. of Animals	Mean ± S.D.	No. of Animals	Mean ± S.D.
0.5	1	70	95	12	16
	2	119		20	
1	1	237	181 ± 66	139	106 ± 39
	2	108		63	
	3	197		116	
2	1	194	286 ± 98	107	158 ± 54
	2	389		215	
	3	276		153	
4	1	426	367 ± 51	215	185 ± 26
	2	332		167	
	3	343		174	
6	1	176	204 ± 36	82	95 ± 17
	2	245		114	
	3	192		90	
8	1	126	158 ± 33	82	103 ± 26
	2	192		125	
	3	157		102	
12	1	222	207	82	84 ± 15
	2	192		71	
				100	
16	1	293	210 ± 72	129	92 ± 32
	2	162		71	
	3	176		77	
24	1	84	105 ± 28	55	69 ± 18
	2	95		63	
	3	137		90	

Corresponds to Figure 17 and Table 26 in the text.

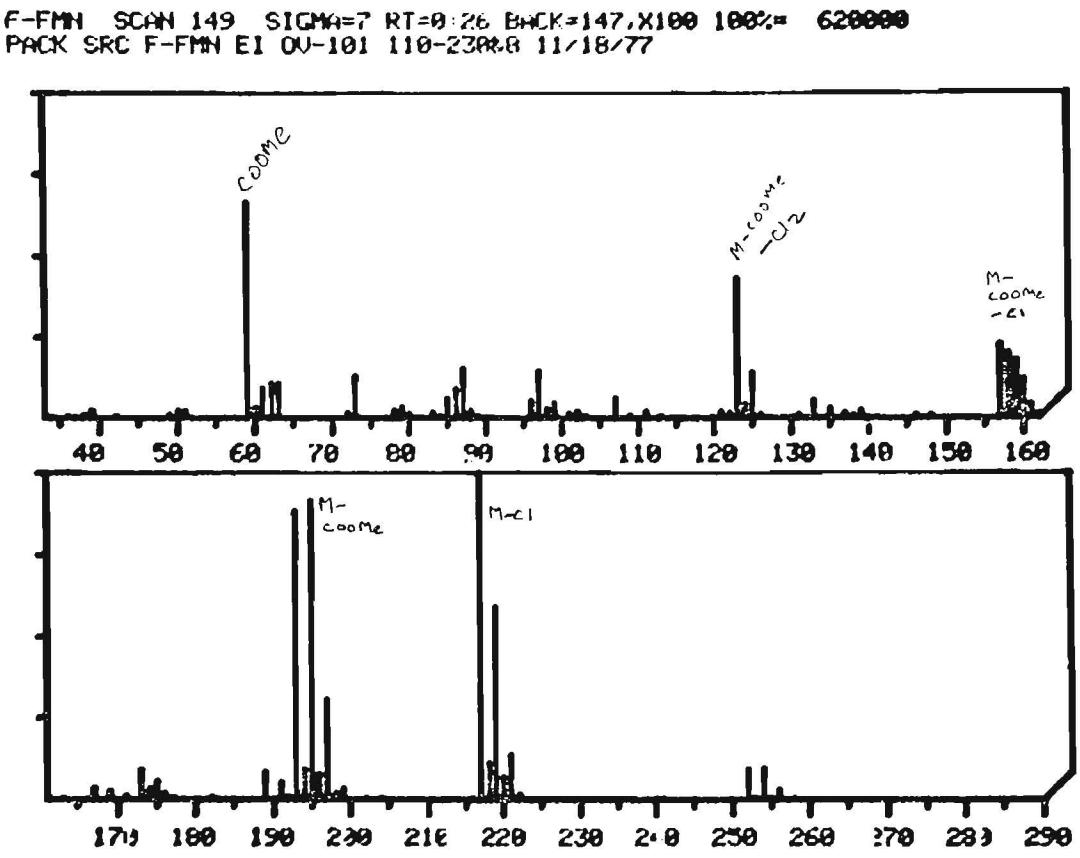

Figure A. Mass Spectra of Fenac Photodegradation Product

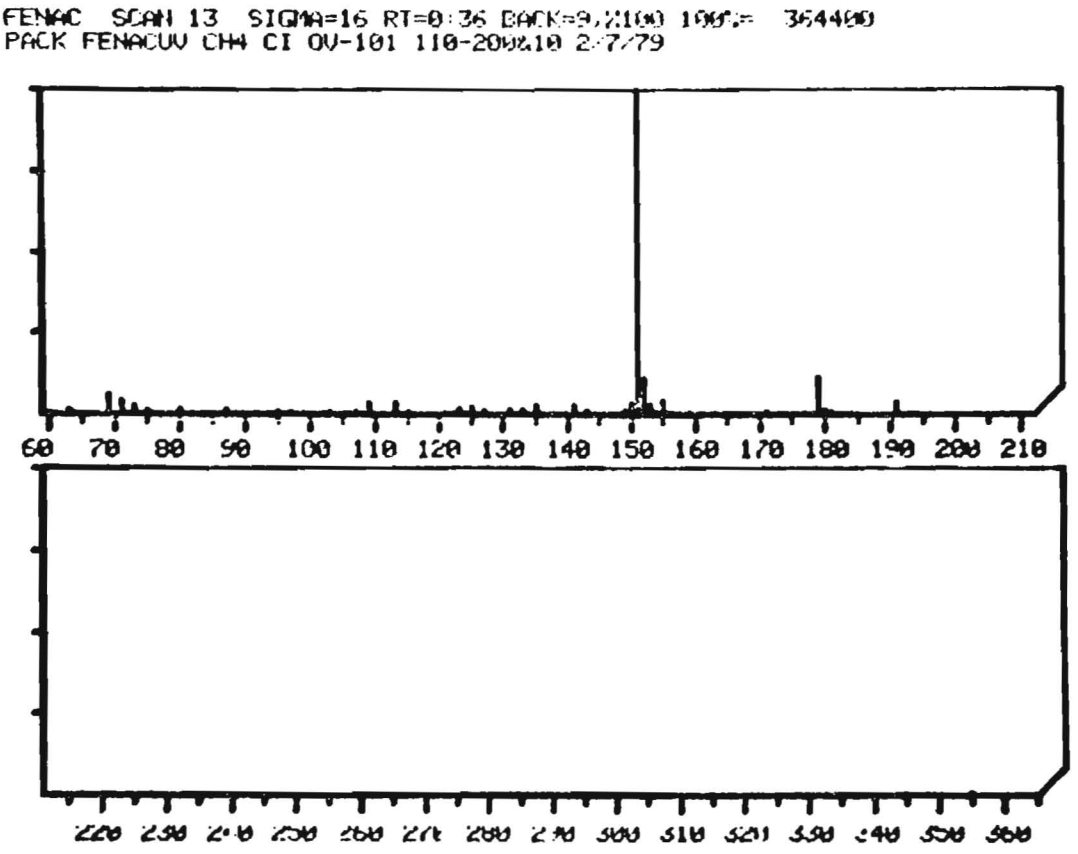

Figure B. Mass Spectra of Fenac Photodegradation Product

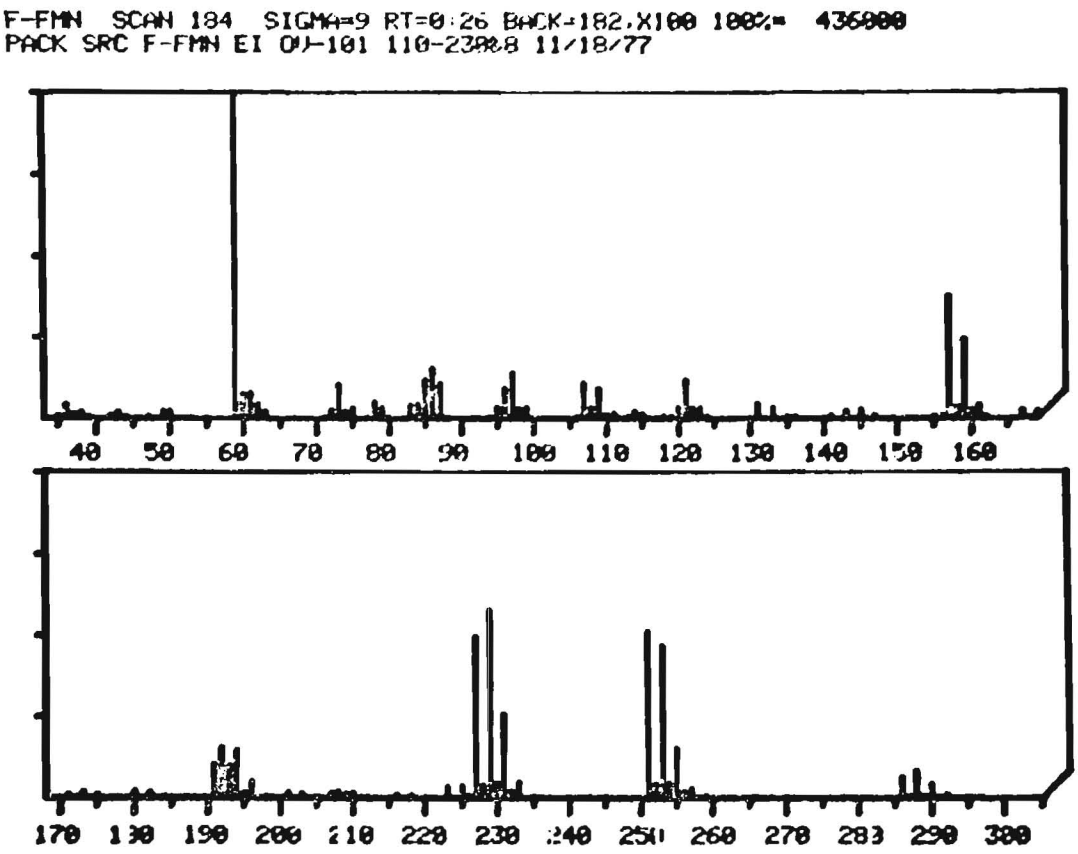

Figure C. Mass Spectra of Fenac Photodegradation Product

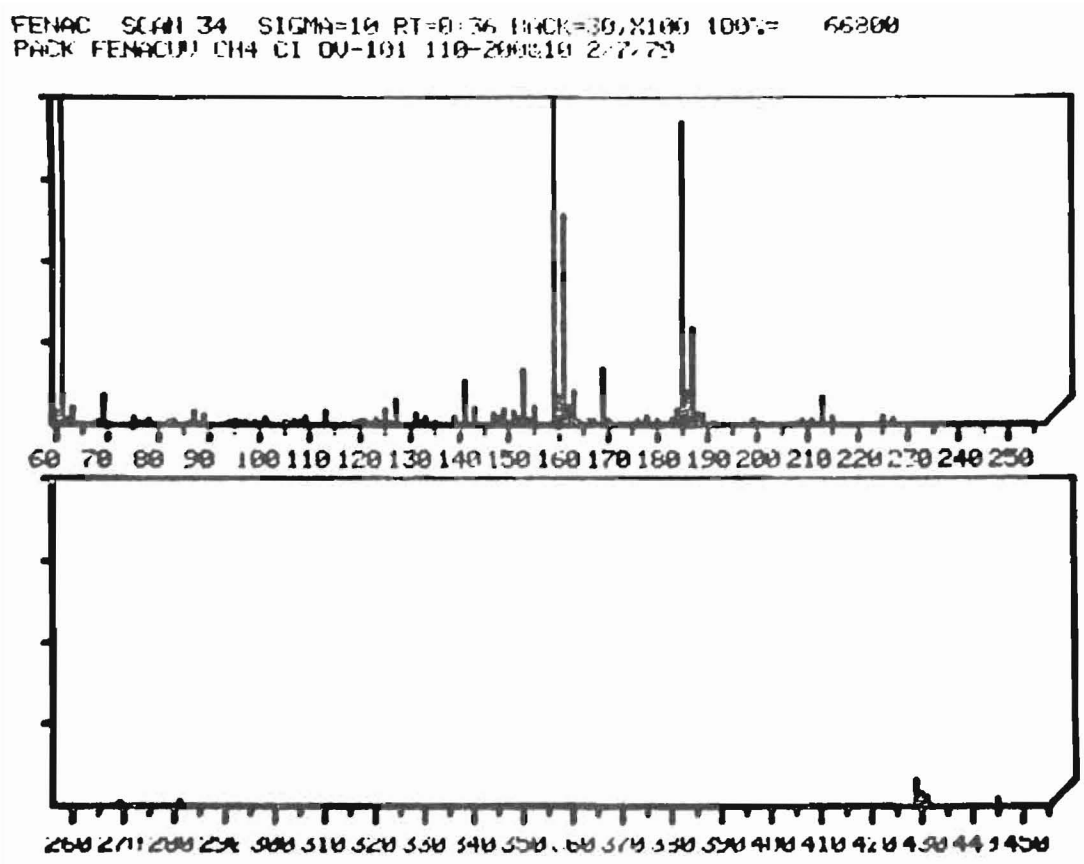

Figure D. Mass Spectra of Fenac Photodegradation Product

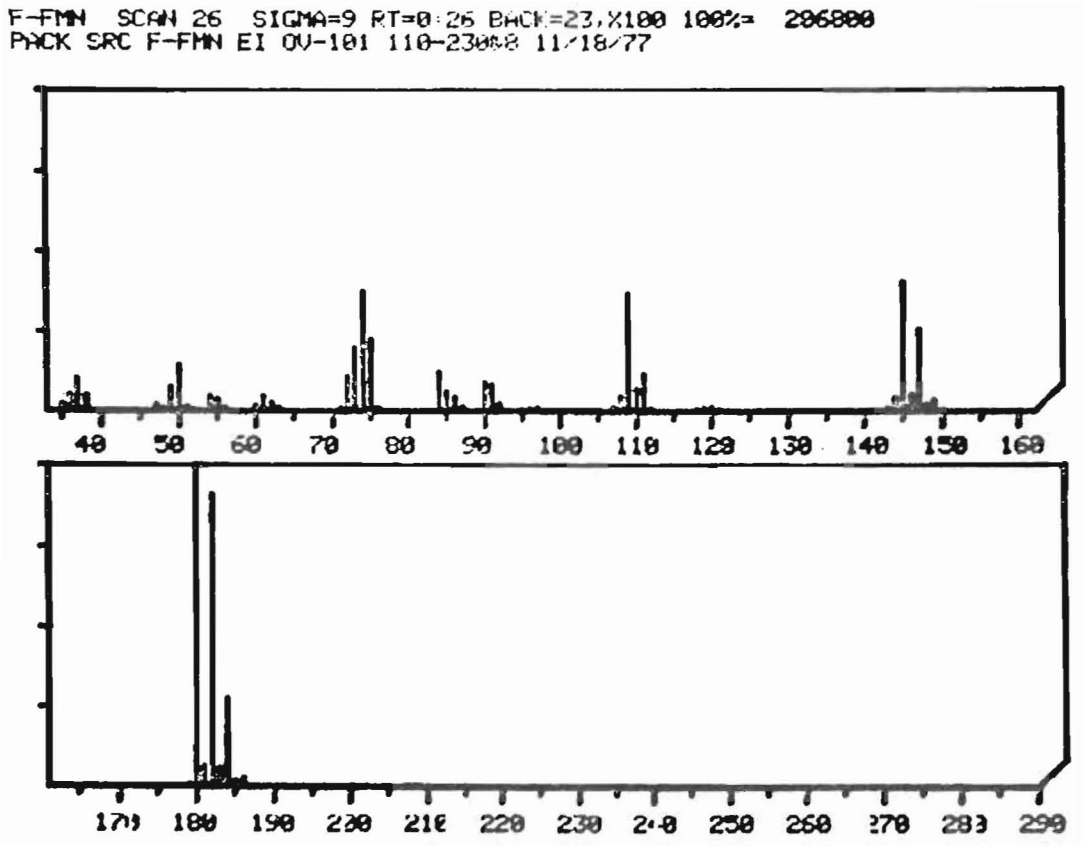

Figure E. Mass Spectra of Fenac Photodegradation Product

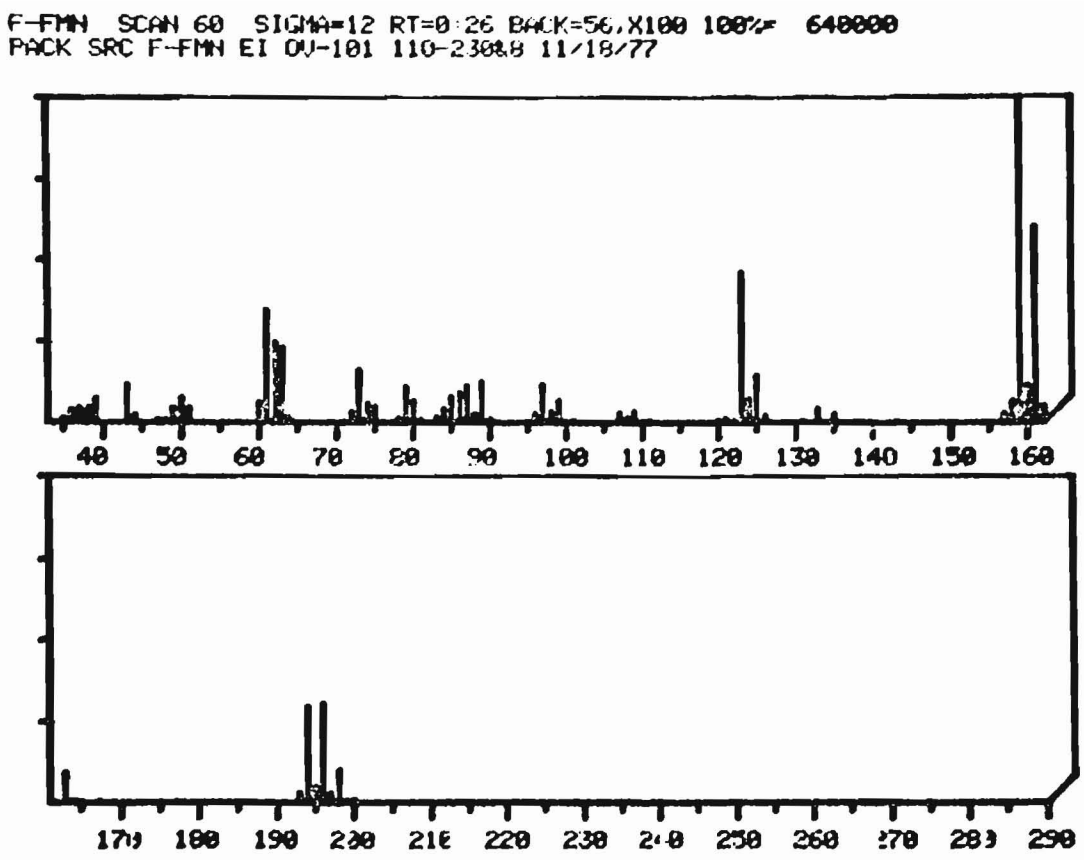

Figure F. Mass Spectra of Fenac Photodegradation Product

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Environmental fate, effects, and health hazards of fenac / by Harish C. Sikka ... [et al]. (Syracuse Research Corporation). -- Vicksburg, Miss. : U.S. Army Engineer Waterways Experiment Station ; Springfield, Va. : available from NTIS, 1982.
85, 35 p. : ill. ; 27 cm. -- (Technical report ; A-82-2)
Cover title.
"February 1982."
Final report.
"Prepared for Office, Chief of Engineers, U.S. Army under Contract No. DACW39-77-C-0021."
"Monitored by Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station."
1. Aquatic ecology. 2. Environmental health. 3. Environmental impact analysis. 4. Herbicides. I. Sikka, Harish C. II. United States. Army. Corps of Engineers. Office of the Chief of Engineers. III. U.S. Army Engineer Waterways Experiment Station. Environmental Laboratory. IV. Series: Technical report (U.S. Army Engineer Waterways Experiment Station) ; A-82-2.
TA7.W34 no.A-82-2